CHAPTER 5: Implantation and Placental Development

Almost immediately after the implantation of the ovum, its trophoblast begins to proliferate and invade the surrounding decidual tissue. As it does so, it breaks through the walls of the maternal capillaries, from which the blood escapes and forms cavities, which are bounded partly by trophoblast and partly by decidua. The maternal blood spaces established in this manner represent the earliest stages of the intervillous blood spaces of the future placenta.

—J. Whitridge Williams (1903)

INTRODUCTION

In 1903, the histopathological and embryological descriptions of ovum implantation and placental development had been extensively studied and described. However, the origins and functions of pregnancy hormones were largely unknown. Indeed, it was another 25 to 30 years before estrogen and progesterone were discovered. In the past 50 years, remarkable strides have followed to uncover the steps of implantation and placental structure and function.

All obstetricians should understand the basic biological steps required for women to successfully achieve pregnancy. Several abnormalities can affect each of these and lead to infertility or pregnancy loss. In most women, spontaneous, cyclical ovulation continues during almost 40 years between menarche and menopause. Without contraception, there are approximately 400 opportunities for pregnancy, namely, the day of ovulation and its few preceding days. This narrow window for fertilization is controlled by tightly regulated production of ovarian steroids. Moreover, these hormones promote optimal endometrial regeneration after menstruation in preparation for the next implantation window.

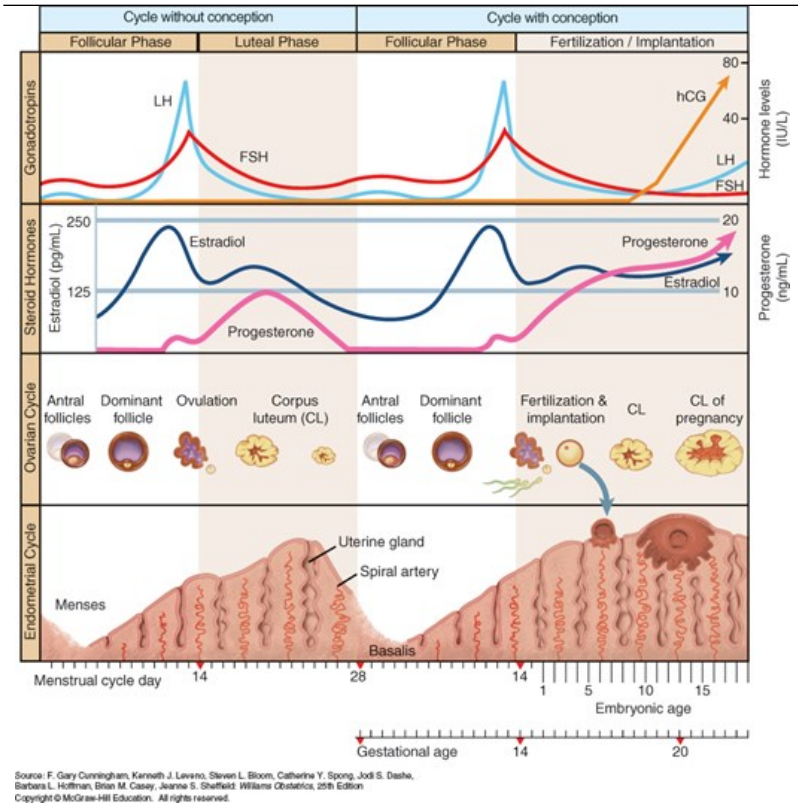
If fertilization occurs, events that begin after blastocyst implantation persist until parturition. These derive from a unique interaction between fetal trophoblasts and the maternal endometrium, which has been transformed into the *decidua*. The ability of a mother and her fetus to coexist as two distinct immunological systems results from endocrine, paracrine, and immunological modification of fetal and maternal tissues in a manner not seen elsewhere. In addition, the placenta mediates a unique fetal–maternal communication system, which creates a hormonal environment that initially maintains pregnancy and eventually initiates events leading to parturition.

OVARIAN–ENDOMETRIAL CYCLE

Predictable, regular, cyclical, and spontaneous ovulatory menstrual cycles are regulated by complex interactions of the hypothalamic-pituitary-ovarian axis. Concurrently, cyclical changes in endometrial histology are faithfully reproduced (Fig. 5-1). Essentials players in this process include follicle-stimulating hormone (FSH) and luteinizing hormone (LH), which are pituitary-derived gonadotropins, and the ovarian sex steroid hormones estrogen and progesterone.

FIGURE 5-1

Gonadotropin control of the ovarian and endometrial cycles. The ovarian-endometrial cycle has been structured as a 28-day cycle. The follicular phase (days 1 to 14) is characterized by rising estrogen levels, endometrial thickening, and selection of the dominant “ovulatory” follicle. During the luteal phase (days 14 to 21), the corpus luteum (CL) produces estrogen and progesterone, which prepare the endometrium for implantation. If implantation occurs, the developing blastocyst begins to produce human chorionic gonadotropin (hCG) and rescues the corpus luteum, thus maintaining progesterone production. FSH = follicle-stimulating hormone; LH = luteinizing hormone.



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The average cycle duration approximates 28 days but ranges from 25 to 32 days, even for a given woman. The follicular or proliferative phase shows considerable phase-length variation. This contrasts with the luteal or secretory postovulatory phase of the cycle, which is remarkably constant at 12 to 14 days.

Ovarian Cycle

Follicular Phase

The human ovary contains 2 million oocytes at birth, and approximately 400,000 follicles are present at puberty onset (Baker, 1963). These are depleted at a rate of approximately 1000 follicles per month until age 35, when this rate accelerates (Faddy, 1992). Only 400 follicles are normally released during female reproductive life. Therefore, more than 99.9 percent of these undergo atresia through a process of cell death termed apoptosis (Gougeon, 1996; Kaipia, 1997).

Follicular development consists of several stages. Primordial follicles undergo gonadotropin-independent recruitment from the resting pool and then progress from primary and secondary follicles to the antral stage. This appears to be controlled by locally produced growth factors. Two members of the transforming growth factor-β family include growth differentiation factor 9 (GDF9) and bone morphogenetic protein 15 (BMP-15), which regulate granulosa cell proliferation and differentiation as primary follicles grow (Trombly, 2009; Yan, 2001). They also stabilize and expand the cumulus oocyte complex in the oviduct (Hreinsson, 2002). These factors are produced by oocytes, suggesting that the early steps in follicular development are, in part, oocyte controlled. As antral follicles develop, surrounding stromal cells are recruited, by a yet-to-be-defined mechanism, to become thecal cells.

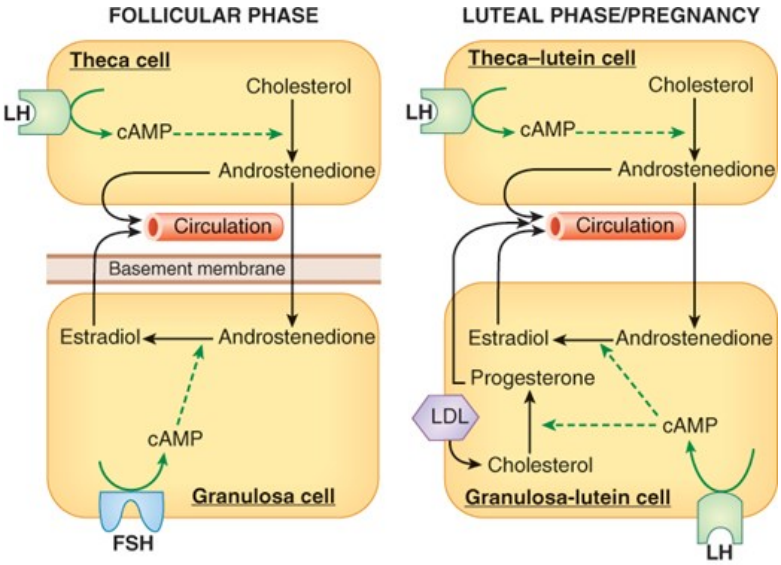
Although not required for early follicular maturation, FSH is required for further development of large antral follicles (Hillier, 2001). During each ovarian cycle, a group of antral follicles, known as a cohort, begins a phase of semisynchronous growth based on their maturation state during the FSH rise in the late luteal phase of the previous cycle. This FSH increase leading to further follicular development is called the *selection window* of the ovarian cycle (Macklon, 2001). Only follicles progressing to this stage develop the capacity to produce estrogen.

During the follicular phase, estrogen levels rise in proportion to growth of a dominant follicle and to the increase in its number of granulosa cells (see Fig. 5-1). These cells are the exclusive site of FSH receptor expression. The elevation of circulating FSH levels during the late luteal phase of the previous cycle stimulates an increase in FSH receptors and subsequently, the ability of cytochrome P450 aromatase within granulosa cells to convert

androstenedione into estradiol. The requirement for thecal cells, which respond to LH, and granulosa cells, which respond to FSH, represents the two-gonadotropin, two-cell hypothesis for estrogen biosynthesis (Short, 1962). As shown in Figure 5-2, FSH induces aromatase and expansion of the antrum of growing follicles. The follicle within the cohort that is most responsive to FSH is likely to be the first to produce estradiol and initiate expression of LH receptors.

FIGURE 5-2

The two-cell, two-gonadotropin principle of ovarian steroid hormone production. During the follicular phase (*left panel*), luteinizing hormone (LH) controls theca cell production of androstenedione, which diffuses into the adjacent granulosa cells and acts as precursor for estradiol biosynthesis. The granulosa cell capacity to convert androstenedione to estradiol is controlled by follicle-stimulating hormone (FSH). After ovulation (*right panel*), the corpus luteum forms and both theca-lutein and granulosa-lutein cells respond to LH. The theca-lutein cells continue to produce androstenedione, whereas granulosa-lutein cells greatly increase their capacity to produce progesterone and to convert androstenedione to estradiol. LH and hCG bind to the same LH-hCG receptor. If pregnancy occurs (*right panel*), human chorionic gonadotropin (hCG) rescues the corpus luteum through their shared LH-hCG receptor. Low-density lipoproteins (LDL) are an important source of cholesterol for steroidogenesis. cAMP = cyclic adenosine monophosphate.



After the appearance of LH receptors, the preovulatory granulosa cells begin to secrete small quantities of progesterone. The preovulatory progesterone secretion, although somewhat limited, is believed to exert positive feedback on the estrogen-primed pituitary to either cause or augment LH release. In addition, during the late follicular phase, LH stimulates thecal cell production of androgens, particularly androstenedione, which are then transferred to the adjacent follicles where they are aromatized to estradiol (see Fig. 5-2). During the early follicular phase, granulosa cells also produce inhibin B, which can feed back on the pituitary to inhibit FSH release (Groome, 1996). As the dominant follicle begins to grow, estradiol and inhibin production rises and results in a decline of follicular-phase FSH. This drop in FSH levels is responsible for the failure of other follicles to reach preovulatory status—the Graafian follicle stage—during any one cycle. Thus, 95 percent of plasma estradiol produced at this time is secreted by the dominant follicle—the one destined to ovulate. Concurrently, the contralateral ovary is relatively inactive.

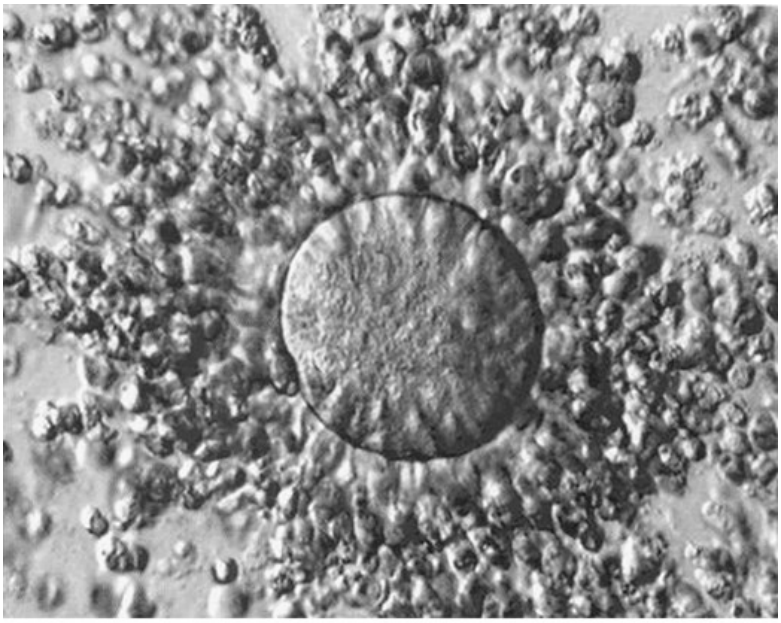
Ovulation

The onset of the gonadotropin surge resulting from increasing estrogen secretion by preovulatory follicles is a relatively precise predictor of ovulation. It occurs 34 to 36 hours before ovum release from the follicle (see Fig. 5-1). LH secretion peaks 10 to 12 hours before ovulation and stimulates resumption of meiosis in the ovum and release of the first polar body. Studies suggest that in response to LH, greater progesterone and prostaglandin production by the cumulus cells, as well as GDF9 and BMP-15 by the oocyte, activates expression of genes critical to formation of a hyaluronan-rich extracellular matrix by the cumulus complex (Richards, 2007). As seen in Figure 5-3, during synthesis of this matrix, cumulus cells lose contact with one another and move outward from the oocyte along the hyaluronan polymer—this process is called expansion. This results in a 20-fold augmentation of

the cumulus complex volume and coincides with an LH-induced remodeling of the ovarian extracellular matrix. These allow release of the mature oocyte and its surrounding cumulus cells through the surface epithelium. Activation of proteases likely plays a pivotal role in weakening the follicular basement membrane and ovulation (Curry, 2006; Ny, 2002).

FIGURE 5-3

An ovulated cumulus–oocyte complex. An oocyte is at the center of the complex. Cumulus cells are widely separated from each other by the hyaluronan-rich extracellular matrix. (Used with permission from Dr. Kevin J. Doody.)



Luteal Phase

Following ovulation, the corpus luteum develops from the remains of the Graafian follicle in a process referred to as *luteinization*. The basement membrane separating the granulosa-lutein and theca-lutein cells breaks down, and by day 2 postovulation, blood vessels and capillaries invade the granulosa cell layer. The rapid neovascularization of the once-avascular granulosa may be due to angiogenic factors that include vascular endothelial growth factor (VEGF) and others produced by theca-lutein and granulosa-lutein cells in response to LH (Albrecht, 2003; Fraser, 2001). During luteinization, these cells undergo hypertrophy and increase their capacity to synthesize hormones.

LH is the primary luteotropic factor responsible for corpus luteum maintenance (Vande Wiele, 1970). Indeed, LH injections can extend the corpus luteum life span in normal women by 2 weeks (Segaloff, 1951).

The hormone secretion pattern of the corpus luteum differs from that of the follicle (see Fig. 5-1). As depicted in Figure 5-2, the greater capacity of granulosa-lutein cells to produce progesterone results from enhanced access to considerably more steroidogenic precursors through blood-borne, low-density lipoprotein (LDL)-derived cholesterol (Carr, 1981a). Ovarian progesterone production peaks at 25 to 50 mg/d during the midluteal phase. With pregnancy, the corpus luteum continues progesterone production in response to placental human chorionic gonadotropin (hCG), which binds to the same receptor as LH.

Estrogen levels follow a more complex pattern of secretion. Specifically, just after ovulation, estrogen levels decline, but then exhibit a secondary rise that reaches a peak production of 0.25 mg/d of 17β-estradiol in the midluteal phase. Toward the end of the luteal phase, estradiol production again drops.

The human corpus luteum is a transient endocrine organ that, in the absence of pregnancy, will rapidly regress 9 to 11 days after ovulation via apoptosis (Vaskivuo, 2002). The mechanisms that control luteolysis, that is, the regression of the corpus luteum, remain unclear. However, it results in

part from dropping levels of circulating LH in the late luteal phase and rising LH insensitivity by luteal cells (Duncan, 1996; Filicori, 1986). The role of other factors is less established. The dramatic drop in circulating estradiol and progesterone levels initiate molecular events that lead to menstruation.

Estrogen and Progesterone Action

Estrogen is the essential hormonal signal on which most events in the normal menstrual cycle depend. They function in many cell types to regulate follicular development, uterine receptivity, and blood flow. The most biologically potent naturally occurring estrogen is 17β-estradiol, which is secreted by granulosa cells of the dominant follicle and luteinized granulosa cells of the corpus luteum. Estradiol action is complex and appears to involve two classic nuclear hormone receptors designated estrogen receptor α (ERα) and β (ERβ) (Katzenellenbogen, 2001). These isoforms are the products of separate genes and can exhibit distinct tissue expression. Both estradiol-receptor complexes act as transcriptional factors that become associated with the estrogen-response element of specific genes. They share a robust activation by estradiol. However, differences in their binding affinities to other estrogens and their cell-specific expression patterns suggest that ERα and ERβ receptors may have both distinct and overlapping function (Saunders, 2005).

Most progesterone actions on the female reproductive tract are mediated through the nuclear hormone receptors, progesterone-receptor type A (PR-A) and B (PR-B). Progesterone enters cells by diffusion, and in responsive tissues it becomes associated with its receptors (Conneely, 2002). Progesterone-receptor isoforms arise from a single gene and regulate transcription of target genes. These receptors have unique actions. When PR-A and PR-B receptors are coexpressed, it appears that PR-A can inhibit PR-B gene regulation. The endometrial glands and stroma appear to have different expression patterns for progesterone receptors that vary during the menstrual cycle (Mote, 1999).

Progesterone can also evoke rapid responses, such as changes in intracellular free calcium levels, which cannot be explained by genomic mechanisms. G-protein–coupled membrane receptors for progesterone have been identified, but their role in the ovarian–endometrial cycle remains to be elucidated (Peluso, 2007).

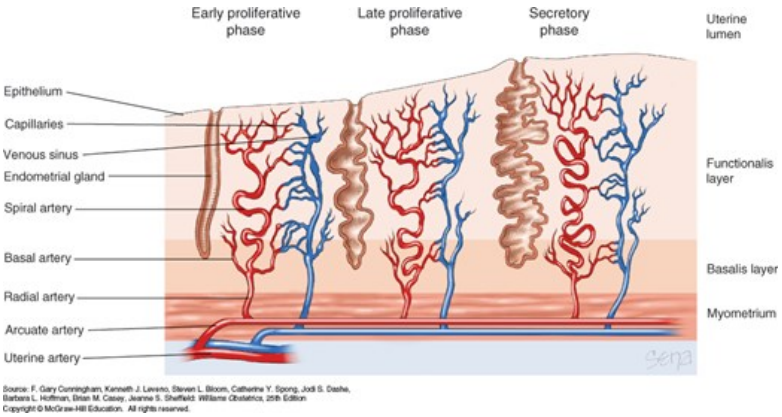
Endometrial Cycle

Proliferative Phase

In the endometrium, epithelial cells line the endometrial glands and are supported by stromal cells. These cells and supplying blood vessels replicate rapidly and cyclically in reproductive-aged women and are regenerated each ovarian–endometrial cycle. The superficial endometrium, termed the *functionalis layer*, is shed and reconstructed from the deeper *basalis layer* (Fig. 5-4). There is no other example in humans of such cyclical shedding and regrowth of an entire tissue.

FIGURE 5-4

The endometrium consists of two layers, the functionalis layer and basalis layer. These are supplied by the spiral and basal arteries, respectively. Numerous glands also span these layers. As the menstrual cycle progresses, greater coiling of the spiral arteries and increased gland folding can be seen. Near the end of the menstrual cycle (day 27), the coiled arteries constrict, deprive blood supply to the functionalis layer, and lead to necrosis and sloughing of this layer.



Fluctuations in estrogen and progesterone levels produce striking effects on the endometrium. Follicular-phase estradiol production is the most important factor in endometrial recovery following menstruation, and both ERα and ERβ receptors are expressed here. Although up to two thirds of the functionalis endometrium is fragmented and shed with menses, reepithelialization begins even before menstrual bleeding has ceased. By the fifth day of the endometrial cycle—fifth day of menses-the epithelial surface of the endometrium has been restored, and revascularization has begun. The preovulatory endometrium is characterized by proliferation of glandular, stromal, and vascular endothelial cells. During the early part of the proliferative phase, the endometrium is usually less than 2 mm thick. The glands are narrow, tubular structures that pursue almost a straight and parallel course from the basalis layer toward the endometrial cavity. Mitotic figures, especially in the glandular epithelium, are identified by the fifth cycle day. Mitotic activity in both epithelium and stroma persists until day 16 to 17, that is, 2 to 3 days after ovulation. Blood vessels are numerous and prominent.

Clearly, reepithelialization and angiogenesis are important to cessation of endometrial bleeding (Chennazhi, 2009; Rogers, 2009). These are dependent on tissue regrowth, which is estrogen regulated. Epithelial cell growth also is regulated in part by epidermal growth factor and transforming growth factor α (TGFα). Stromal cells proliferate through paracrine and autocrine actions of estrogen and greater local levels of fibroblast growth factor-9 (Tsai, 2002). Estrogens also raise local production of VEGF, which causes angiogenesis through vessel elongation in the basalis (Gargett, 2001; Sugino, 2002).

By the late proliferative phase, the endometrium thickens from both glandular hyperplasia and augmented stromal ground substance, which is edema and proteinaceous material. The loose stroma is especially prominent, and the glands in the functionalis layer are widely separated. This is compared with those of the basalis layer, in which the glands are more crowded and the stroma is denser.

At midcycle, as ovulation nears, glandular epithelium becomes taller and pseudostratified. The surface epithelial cells acquire numerous microvilli, which increase epithelial surface area, and develop cilia, which move endometrial secretions during the secretory phase (Ferenczy, 1976).

Secretory Phase

After ovulation, the estrogen-primed endometrium responds to rising progesterone levels in a highly predictable manner. By day 17, glycogen accumulates in the basal portion of glandular epithelium, creating subnuclear vacuoles and pseudostratification. These changes likely result from direct progesterone action through receptors expressed in glandular cells (Mote, 2000). On day 18, vacuoles move to the apical portion of the secretory nonciliated cells. By day 19, these cells begin to secrete glycoprotein and mucopolysaccharide contents into the gland lumen (Hafez, 1975). Glandular cell mitosis ceases with secretory activity due to rising progesterone levels, which antagonize the mitotic effects of estrogen. Estradiol action also diminishes because of glandular expression of the type 2 isoform of 17β-hydroxysteroid dehydrogenase. This converts estradiol to the less active estrone (Casey, 1996). On cycle days 21 to 24, the stroma becomes edematous. Next, on days 22 to 25, stromal cells surrounding the spiral arterioles begin to enlarge, and stromal mitosis becomes apparent. Days 23 to 28 are characterized by predecidual cells, which surround spiral arterioles.

Between days 22 and 25, the secretory-phase endometrium undergoes striking changes associated with predecidual transformation of the upper two thirds of the functionalis layer. The glands exhibit extensive coiling, and luminal secretions become visible. Changes within the endometrium also can mark the so-called window of implantation seen on days 20 to 24. Epithelial surface cells show fewer microvilli and cilia, but luminal protrusions appear on the apical cell surface (Nikas, 2003). These pinopodes help prepare for blastocyst implantation. They also coincide with changes in the surface glycocalyx that allow acceptance of a blastocyst (Aplin, 2003).

Another highlight of the secretory phase is the continuing growth and development of the spiral arteries. These vessels arise from the radial arteries, which are myometrial branches of the arcuate and, ultimately, uterine vessels (see Fig. 5-4). The morphological and functional properties of the spiral arteries are unique and essential to blood flow changes seen during menstruation or implantation. During endometrial growth, spiral arteries lengthen at a rate appreciably greater than the rate of endometrial tissue thickening. This growth discordance obliges even greater coiling. Spiral artery development reflects a marked induction of angiogenesis, with widespread vessel sprouting and extension. Such rapid angiogenesis is regulated, in part, through estrogen- and progesterone-regulated synthesis of VEGF (Ancelin, 2002; Chennazhi, 2009).

Menstruation

The midluteal–secretory phase of the endometrial cycle is a critical branch point in endometrial development and differentiation. With corpus luteum rescue and continued progesterone secretion, the endometrium is transformed into the decidua. With luteolysis and declining luteal progesterone production, events leading to menstruation are initiated (Critchley, 2006; Thiruchelvam, 2013).

In the late premenstrual-phase endometrium, the stroma is invaded by neutrophils to create a pseudoinflammatory appearance. These cells infiltrate primarily on the day or two immediately preceding menses onset. The endometrial stromal and epithelial cells produce interleukin-8 (IL-8), a chemotactic–activating factor for neutrophils (Arici, 1993). Similarly, monocyte chemotactic protein-1 (MCP-1) is synthesized by endometrium and promotes monocyte recruitment (Arici, 1995).

Leukocyte infiltration is considered key to both endometrial extracellular matrix breakdown and repair of the functionalis layer. The term "inflammatory tightrope" refers to the ability of macrophages to assume phenotypes that vary from proinflammatory and phagocytic to immunosuppressive and reparative. These are likely relevant to menstruation, in which tissue breakdown and restoration occur simultaneously (Evans, 2012; Maybin, 2015). Invading leukocytes secrete enzymes that are members of the matrix metalloprotease (MMP) family. These add to the proteases already produced by endometrial stromal cells and effectively initiate matrix degradation. During menses as tissue shedding is completed, microenvironment-regulated changes in macrophage phenotype then promote repair and resolution (Evans, 2012; Thiruchelvam, 2013).

The classic study by Markee (1940) described tissue and vascular alterations in endometrium before menstruation. With endometrial regression, spiral artery coiling becomes sufficiently severe that resistance to blood flow rises to cause endometrial hypoxia. Resultant stasis is the primary cause of endometrial ischemia and tissue degeneration. Intense spiral artery vasoconstriction precedes menstruation and also serves to limit menstrual blood loss.

Prostaglandins play a key role in the events leading to menstruation that include vasoconstriction, myometrial contractions, and upregulation of proinflammatory responses (Abel, 2002). Large amounts of prostaglandins are present in menstrual blood. Painful menstruation is common and likely caused by myometrial contractions and uterine ischemia. This response is believed to be mediated by prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)-induced spiral artery vasoconstriction, which render the uppermost endometrial zones hypoxic. The hypoxic environment is a potent inducer of angiogenesis and vascular permeability factors such as VEGF.

Progesterone withdrawal increases expression of cyclooxygenase 2 (COX-2), also called prostaglandin synthase 2, to synthesize prostaglandins. Withdrawal also lowers expression of 15-hydroxyprostaglandin dehydrogenase (PGDH), which degrades prostaglandins (Casey, 1980, 1989). The net result is higher prostaglandin production by endometrial stromal cells and greater prostaglandin-receptor density on blood vessels and surrounding cells.

Actual menstrual bleeding follows rupture of spiral arterioles and consequent hematoma formation. With a hematoma, the superficial endometrium is distended and ruptures. Subsequently, fissures develop in the adjacent functionalis layer, and blood and tissue fragments are sloughed. Hemorrhage stops with arteriolar constriction. Changes that accompany partial tissue necrosis also serve to seal vessel tips.

The endometrial surface is restored by growth of flanges, or collars, that form the everted free ends of the endometrial glands (Markee, 1940). These flanges rapidly grow in diameter, and epithelial continuity is reestablished by fusion of the edges of these sheets of migrating cells.

DECIDUA

This is a specialized, highly modified endometrium of pregnancy. It is essential for *hemochorial placentation*, that is, one in which maternal blood contacts trophoblasts. This relationship requires trophoblast invasion, and considerable research has focused on the interaction between decidual cells and invading trophoblasts. *Decidualization*, that is, transformation of proliferating endometrial stromal cells into specialized secretory cells, is dependent on estrogen, progesterone, androgens, and factors secreted by the implanting blastocyst (Gibson, 2016). The decidua produces factors that regulate endometrial receptivity and modulate immune and vascular cell functions within the maternal–fetal microenvironment. The special relationship existing between the decidua and the invading trophoblasts ensures success of the pregnancy semiallograft yet seemingly defies the laws of transplantation immunology.

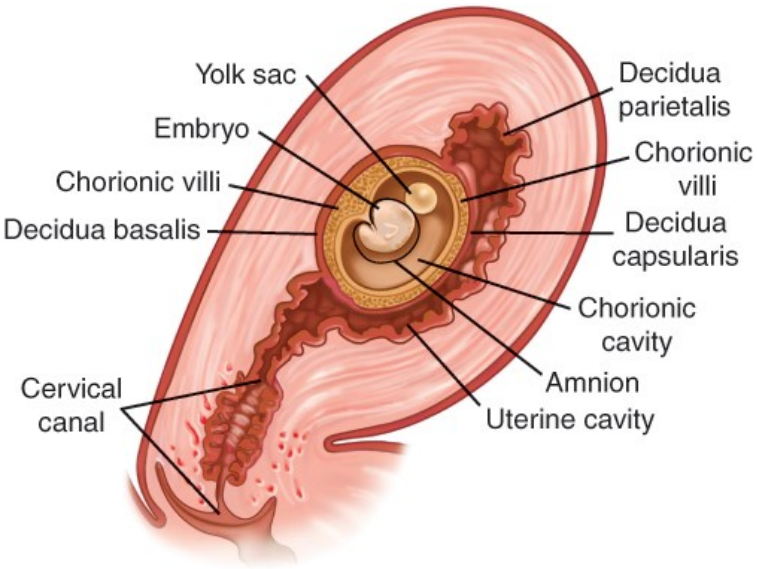
Decidual Structure

The decidua is classified into three parts based on anatomical location. Decidua directly beneath blastocyst implantation is modified by trophoblast invasion and becomes the *decidua basalis*. The *decidua capsularis* overlies the enlarging blastocyst and initially separates the conceptus from the rest of the uterine cavity (Fig. 5-5). This portion is most prominent during the second month of pregnancy and consists of stromal decidual cells covered by a single layer of flattened epithelial cells. Internally, it contacts the avascular, extraembryonic fetal membrane—the chorion laeve. The remainder of the

uterus is lined by *decidua parietalis*. During early pregnancy, there is a space between the decidua capsularis and parietalis because the gestational sac does not fill the entire uterine cavity. The gestation sac is the extraembryonic coelom and also called the chorionic cavity. By 14 to 16 weeks' gestation, the expanding sac has enlarged to completely fill the uterine cavity. The resulting apposition of the decidua capsularis and parietalis creates the *decidua vera*, and the uterine cavity is functionally obliterated.

FIGURE 5-5

Three portions of the decidua—the basalis, capsularis, and parietalis—are illustrated.



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In early pregnancy, the decidua begins to thicken, eventually attaining a depth of 5 to 10 mm. With magnification, furrows and numerous small openings, representing the mouths of uterine glands, can be detected. Later in pregnancy, the decidua becomes thinner, presumably because of pressure exerted by the expanding uterine contents.

The decidua parietalis and basalis are composed of three layers. There is a surface or compact zone—*zona compacta*; a middle portion or spongy zone—*zona spongiosa*—with remnants of glands and numerous small blood vessels; and a basal zone—*zona basalis*. The zona compacta and spongiosa together form the *zona functionalis*. The basal zone remains after delivery and gives rise to new endometrium.

In human pregnancy, the decidual reaction is completed only with blastocyst implantation. Predecidual changes, however, commence first during the midluteal phase in endometrial stromal cells adjacent to the spiral arteries and arterioles. Thereafter, they spread in waves throughout the uterine endometrium and then from the implantation site. The endometrial stromal cells enlarge to form polygonal or round decidual cells. The nuclei become vesicular, and the cytoplasm becomes clear, slightly basophilic, and surrounded by a translucent membrane.

As a consequence of implantation, the blood supply to the decidua capsularis is lost as the embryo–fetus grows. Blood supply to the decidua parietalis through spiral arteries persists. These arteries retain a smooth-muscle wall and endothelium and thereby remain responsive to vasoactive agents.

In contrast, the spiral arterial system that supplies the decidua basalis and ultimately the placental intervillous space is altered remarkably. These spiral arterioles and arteries are invaded by trophoblasts, and during this process, the vessel walls in the basalis are destroyed. Only a shell without smooth muscle or endothelial cells remains. Importantly, as a result, these vascular conduits of maternal blood—which become the uteroplacental vessels—are unresponsive to vasoactive agents. Conversely, the fetal chorionic vessels, which transport blood between the placenta and the fetus, contain smooth muscle and thus do respond to vasoactive agents.

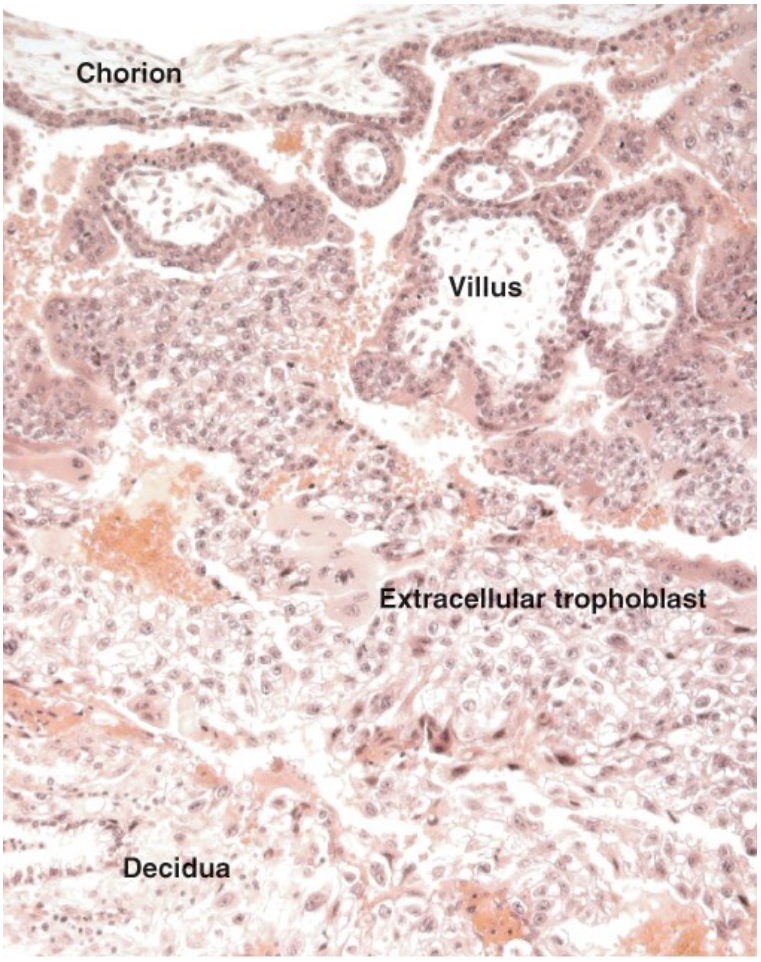
Decidual Histology

Early in pregnancy, the zona spongiosa of the decidua consists of large distended glands, often exhibiting marked hyperplasia and separated by minimal stroma. At first, the glands are lined by typical cylindrical uterine epithelium with abundant secretory activity that contributes to blastocyst nourishment. With advanced pregnancy, the glandular elements largely disappear.

The decidua basalis contributes to formation of the placental basal plate (Fig. 5-6). The spongy zone of the decidua basalis consists mainly of arteries and widely dilated veins, and by term, glands have virtually disappeared. Also, the decidua basalis is invaded by many interstitial trophoblasts and trophoblastic giant cells. Although most abundant in the decidua, the giant cells commonly penetrate the upper myometrium. Their number and invasiveness can be so extensive as to resemble choriocarcinoma.

FIGURE 5-6

Section through a junction of chorion, villi, and decidua basalis in early first-trimester pregnancy. (Used with permission from Dr. Kurt Benirschke.)



The *Nitabuch layer* is a zone of fibrinoid degeneration in which invading trophoblasts meet the decidua basalis. If the decidua is defective, as in placenta accreta, the Nitabuch layer is usually absent (Chap. 41, Classification). There is also a more superficial, but inconsistent, deposition of fibrin—*Rohr stria*—at the bottom of the intervillous space and surrounding the anchoring villi. Decidual necrosis is a normal phenomenon in the first and probably second trimesters (McCombs, 1964). Thus, necrotic decidua obtained through curettage after spontaneous abortion in the first trimester should not necessarily be interpreted as either a cause or an effect of the pregnancy loss.

Both decidua types contain numerous cell groups whose composition varies with gestational stage (Loke, 1995). The primary cellular components are the true decidual cells, which differentiated from the endometrial stromal cells, and numerous maternal bone marrow-derived cells. Accumulation of lymphocytes with unique properties at the maternal–fetal interface is essential to evoke tolerance mechanisms that prevent maternal immune

rejection of the fetus. These include regulatory T cells, decidual macrophages, and decidual natural killer cells. Collectively, these cells not only provide immunotolerance but also play an important role in trophoblast invasion and vasculogenesis (PrabhuDas, 2015).

Decidual Prolactin

In addition to placental development, the decidua potentially provides other functions. The decidua is the source of prolactin, which is present in enormous amounts in amnionic fluid (Golander, 1978; Riddick, 1979). Decidual prolactin is a product of the same gene that encodes for anterior pituitary prolactin, but the exact physiological role of decidual prolactin is unknown. Notably, decidual prolactin is not to be confused with placental lactogen (hPL), which is produced only by syncytiotrophoblast.

Prolactin preferentially enters amnionic fluid, and little enters maternal blood. Consequently, prolactin levels in amnionic fluid are extraordinarily high and may reach 10,000 ng/mL at 20 to 24 weeks' gestation (Tyson, 1972). This compares with fetal serum levels of 350 ng/mL and maternal serum levels of 150 to 200 ng/mL. As a result, decidual prolactin is a classic example of paracrine function between maternal and fetal tissues.

IMPLANTATION AND EARLY TROPHOBLAST FORMATION

The fetus is dependent on the placenta for pulmonary, hepatic, and renal functions. These are accomplished through the anatomical relationship of the placenta and its uterine interface. In overview, maternal blood spurts from uteroplacental vessels into the placental intervillous space and bathes the outer syncytiotrophoblast. This allows exchange of gases, nutrients, and other substances with fetal capillary blood within the core of each villus. Thus, fetal and maternal blood does not normally mix in this hemochorial placenta. A paracrine system also links mother and fetus through the anatomical and biochemical juxtaposition of the maternal decidua parietalis and the extraembryonic chorion laeve, which is fetal. This is an extraordinarily important arrangement for communication between fetus and mother and for maternal immunological acceptance of the conceptus (Guzeloglu-Kayisli, 2009).

Fertilization

With ovulation, the secondary oocyte and adhered cells of the cumulus–oocyte complex are freed from the ovary. Although technically this mass of cells is released into the peritoneal cavity, the oocyte is quickly engulfed by the fallopian tube infundibulum. Further transport through the tube is accomplished by directional movement of cilia and tubal peristalsis. Fertilization, which normally occurs in the oviduct, must take place within a few hours, and no more than a day after ovulation. Because of this narrow window, spermatozoa must be present in the fallopian tube at the time of oocyte arrival. Almost all pregnancies result when intercourse occurs during the 2 days preceding or on the day of ovulation.

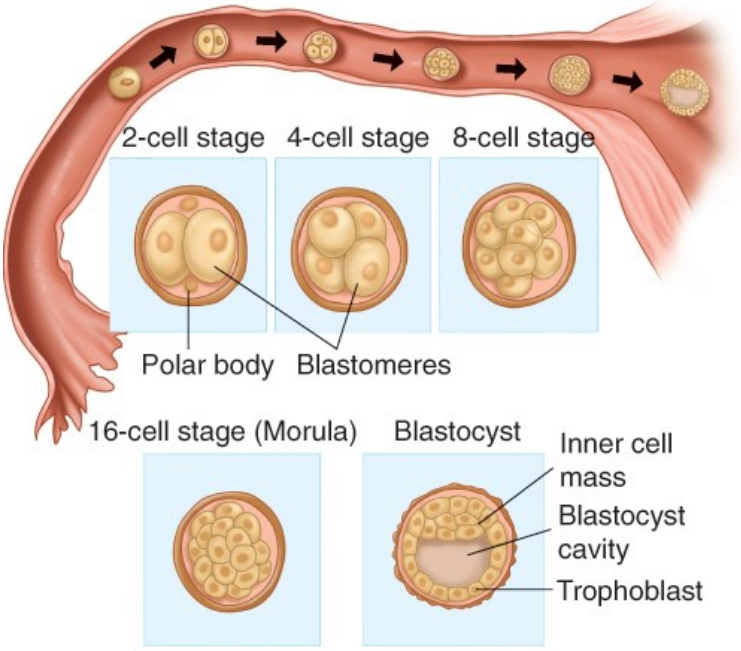
Fertilization is highly complex. Molecular mechanisms allow spermatozoa to pass between follicular cells; through the zona pellucida, which is a thick glycoprotein layer surrounding the oocyte cell membrane; and into the oocyte cytoplasm. Fusion of the two nuclei and intermingling of maternal and paternal chromosomes creates the *zygote*.

Early human development is described by days or weeks postfertilization, that is, postconceptional. By contrast, in most chapters of this book, clinical pregnancy dating is calculated from the first day of the last menstrual period (LMP). Thus, 1 week postfertilization corresponds to approximately 3 weeks from the LMP in women with regular 28-day cycles. As an example, 8 weeks' gestation refers to 8 completed weeks following the LMP.

After fertilization, the zygote—a diploid cell with 46 chromosomes—undergoes cleavage, and zygote cells produced by this division are called *blastomeres* (Fig. 5-7). In the two-cell zygote, the blastomeres and polar body continue to be surrounded by the zona pellucida. The zygote undergoes slow cleavage for 3 days while still remaining in the fallopian tube. As the blastomeres continue to divide, a solid mulberry-like ball of cells—the *morula*—is produced. The morula enters the uterine cavity approximately 3 days after fertilization. Gradual accumulation of fluid between the morula cells leads to formation of the early *blastocyst*.

FIGURE 5-7

Zygote cleavage and blastocyst formation. The morula period begins at the 12- to 16-cell stage and ends when the blastocyst forms, which occurs when there are 50 to 60 blastomeres present. The polar bodies, shown in the 2-cell stage, are small nonfunctional cells that soon degenerate.



Source: F. Gary Cunningham, Kenneth J. Leveno, Steven L. Bloom, Catherine Y. Spong, Jodi S. Dashe, Barbara L. Hoffman, Brian M. Casey, Jeanne S. Sheffield: *Williams Obstetrics*, 25th Edition

Blastocyst

As early as 4 to 5 days after fertilization, the 58-cell blastula differentiates into five embryo-producing cells—the *inner cell mass* (see Fig. 5-7). The remaining 53 outer cells, called the *trophectoderm,* are destined to form *trophoblasts* (Hertig, 1962).

Interestingly, the 107-cell blastocyst is found to be no larger than the earlier cleavage stages, despite the accumulated fluid within the blastocyst cavity. At this stage, the eight formative, embryo-producing cells are surrounded by 99 trophoblastic cells. And, the blastocyst is released from the zona pellucida secondary to secretion of specific proteases from the secretory-phase endometrial glands (O'Sullivan, 2002).

Release from the zona pellucida allows blastocyst-produced cytokines and hormones to directly influence endometrial receptivity (Lindhard, 2002). IL-1α and IL-1β are secreted by the blastocyst, and these cytokines likely directly influence the endometrium. Embryos also have been shown to secrete hCG, which may influence endometrial receptivity (Licht, 2001; Lobo, 2001). The receptive endometrium is thought to respond by producing leukemia inhibitory factor (LIF), follistatin, and colony-stimulating factor-1 (CSF-1). LIF and follistatin activate signaling pathways that collectively inhibit proliferation and promote differentiation of the endometrial epithelia and stroma to enable uterine receptivity (Rosario, 2016b). At the maternal–fetal interface, CSF-1 has proposed immunomodulatory actions and proangiogenic actions that are required for implantation (Rahmati, 2015).

Implantation

Six or 7 days after fertilization, the blastocyst implants into the uterine wall. This process can be divided into three phases: (1) apposition—initial contact of the blastocyst to the uterine wall; (2) adhesion—increased physical contact between the blastocyst and decidua; and (3) invasion—penetration and invasion of syncytiotrophoblast and cytotrophoblasts into the decidua, inner third of the myometrium, and uterine vasculature.

Successful implantation requires a receptive endometrium appropriately primed with estrogen and progesterone by the corpus luteum. Such uterine receptivity is limited to days 20 to 24 of the cycle. Adherence is mediated by cell-surface receptors at the implantation site that interact with blastocyst receptors (Carson, 2002; Lessey, 2002; Lindhard, 2002). If the blastocyst approaches the endometrium after cycle day 24, the potential for adhesion is diminished because antiadhesive glycoprotein synthesis prevents receptor interactions (Navot, 1991).

At the time of its interaction with the endometrium, the blastocyst is composed of 100 to 250 cells. The blastocyst loosely adheres to the decidua by apposition. This most commonly occurs on the upper posterior uterine wall. Attachment of the blastocyst trophectoderm to the decidual surface by apposition and adherence appears to be closely regulated by paracrine interactions between these two tissues.

Successful endometrial blastocyst adhesion involves modification in expression of cellular adhesion molecules (CAMs). The integrins—one of four families of CAMs—are cell-surface receptors that mediate cell adhesion to extracellular matrix proteins (Lessey, 2002). Endometrial integrins are hormonally regulated, and a specific set of integrins is expressed at implantation (Lessey, 1995). Recognition-site blockade of integrins needed for binding will prevent blastocyst attachment (Kaneko, 2013).

Trophoblast Development

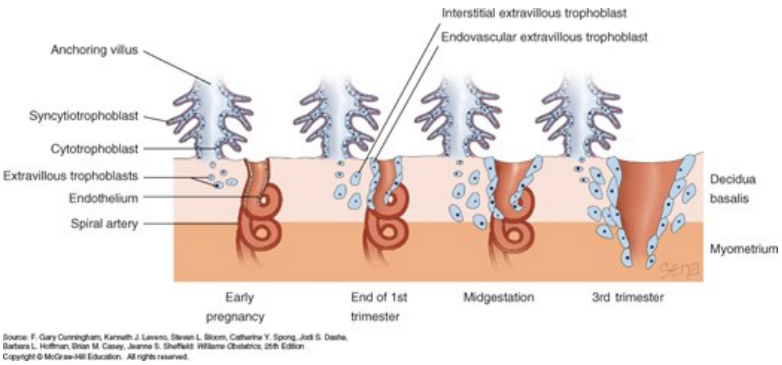
Human placental formation begins with the trophectoderm, which gives rise to a trophoblast cell layer encircling the blastocyst. From then until term, trophoblasts play a critical part at the fetal–maternal interface. Trophoblasts exhibit the most variable structure, function, and developmental pattern of all placental components. Their invasiveness promotes implantation, their nutritional role for the conceptus is reflected in their name, and their endocrine organ function is essential to maternal physiological adaptations and to pregnancy maintenance.

By the eighth day postfertilization, after initial implantation, trophoblasts have differentiated into an outer multinucleated syncytium—primitive *syncytiotrophoblast*, and an inner layer of primitive mononuclear cells—*cytotrophoblasts*. The latter are germinal cells for the syncytium. As cytotrophoblasts proliferate, their cell walls disappear, and the cells fuse to add to the expanding outer layer of syncytiotrophoblast. Each cytotrophoblast has a well-demarcated cell border, a single nucleus, and ability to undergo DNA synthesis and mitosis (Arnholdt, 1991). These are lacking in the syncytiotrophoblast, which provides transport functions of the placenta. It is so named because instead of individual cells, it has an amorphous cytoplasm without cell borders, nuclei that are multiple and diverse in size and shape, and a continuous syncytial lining. This configuration aids transport.

After implantation is complete, trophoblasts further differentiate along two main pathways, giving rise to villous and extravillous trophoblasts. As shown in Figure 5-8, both have distinct functions (Loke, 1995). *Villous trophoblasts* generate chorionic villi, which primarily transport oxygen, nutrients, and other compounds between the fetus and mother. *Extravillous trophoblasts* migrate into the decidua and myometrium and also penetrate maternal vasculature, thus coming into contact with various maternal cell types (Pijnenborg, 1994). Extravillous trophoblasts are further classified as *interstitial trophoblasts* and *endovascular trophoblasts*. The interstitial trophoblasts invade the decidua and eventually penetrate the myometrium to form placental-bed giant cells. These trophoblasts also surround spiral arteries. The endovascular trophoblasts penetrate the spiral artery lumens (Pijnenborg, 1983). These are both discussed in greater detail in subsequent sections.

FIGURE 5-8

Extravillous trophoblasts are found outside the villus and can be subdivided into endovascular and interstitial categories. Endovascular trophoblasts invade and transform spiral arteries during pregnancy to create low-resistance blood flow that is characteristic of the placenta. Interstitial trophoblasts invade the decidua and surround spiral arteries.



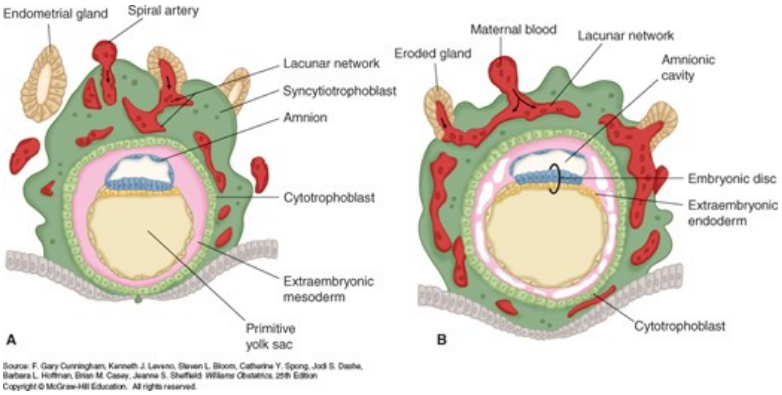
Early Invasion

After gentle erosion between epithelial cells of the surface endometrium, invading trophoblasts burrow deeper. At 9 days of development, the blastocyst wall facing the uterine lumen is a single layer of flattened cells. By the 10th day, the blastocyst becomes totally encased within the endometrium (Fig. 5-9). The blastocyst wall opposite the uterine lumen is thicker and comprises two zones—the trophoblasts and the embryo-forming inner cell mass. As early as 7½ days postfertilization, the inner cell mass or embryonic disc differentiates into a thick plate of primitive ectoderm and an

underlying layer of endoderm. Some small cells appear between the embryonic disc and the trophoblasts and enclose a space that will become the amnionic cavity.

FIGURE 5-9

Drawing of sections through implanted blastocysts. **A.** At 10 days. **B.** At 12 days after fertilization. This stage is characterized by the intercommunication of the lacunae filled with maternal blood. Note in **(B)** that large cavities have appeared in the extraembryonic mesoderm, forming the beginning of the extraembryonic coelom. Also note that extraembryonic endodermal cells have begun to form on the inside of the primitive yolk sac. (Redrawn and adapted from Moore KL, Persaud, TV, Torchia, MG (eds): The Developing Human. Clinically Oriented Embryology, 9th edition, Philadelphia, Saunders, 2013.)



Extraembryonic mesenchyme first appears as groups of isolated cells within the blastocyst cavity, and later this mesoderm completely lines the cavity. Spaces form and then fuse within the extraembryonic mesoderm to form the chorionic cavity (extraembryonic coelom). The *chorion* is composed of trophoblasts and mesenchyme. Some mesenchymal cells eventually will condense to form the body stalk. This stalk joins the embryo to the nutrient chorion and later develops into the umbilical cord. The body stalk can be recognized at an early stage at the caudal end of the embryonic disc (Fig. 7-3).

As the embryo enlarges, more maternal decidua basalis is invaded by syncytiotrophoblast. Beginning approximately 12 days after conception, the syncytiotrophoblast is permeated by a system of intercommunicating channels called trophoblastic lacunae. After invasion of superficial decidual capillary walls, lacunae become filled with maternal blood. At the same time, the decidual reaction intensifies in the surrounding stroma. This is characterized by decidual stromal cell enlargement and glycogen storage.

Chorionic Villi

With deeper blastocyst invasion into the decidua, solid primary villi arise from buds of cytotrophoblasts that protrude into the primitive syncytium before 12 days postfertilization. Primary villi are composed of a cytotrophoblast core covered by syncytiotrophoblast. As the lacunae join, a complicated labyrinth is formed that is partitioned by these solid cytotrophoblastic columns. The trophoblast-lined channels form the intervillous space, and the solid cellular columns form the *primary villous stalks.*

Beginning on approximately the 12th day after fertilization, mesenchymal cords derived from extraembryonic mesoderm invade the solid trophoblast columns. These form *secondary villi.* Once angiogenesis begins in the mesenchymal cords, *tertiary villi* are formed. Although maternal venous sinuses are tapped early in implantation, maternal arterial blood does not enter the intervillous space until around day 15. By approximately the 17th day, however, fetal blood vessels are functional, and a placental circulation is established. The fetal–placental circulation is completed when the blood vessels of the embryo are connected with chorionic vessels. In some villi, angiogenesis fails from lack of circulation. They can be seen normally, but the most striking exaggeration of this process is seen with hydatidiform mole (Fig. 20-1).

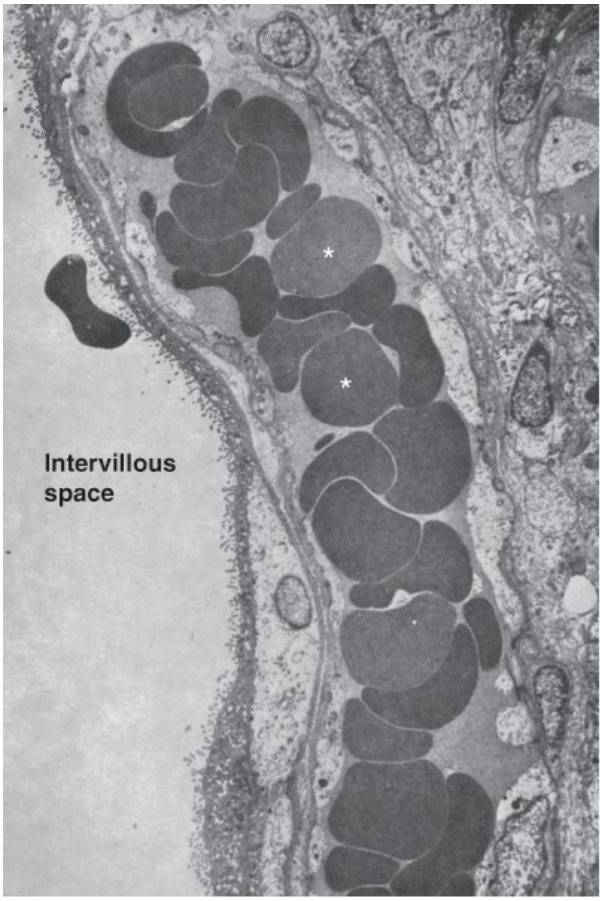
Villi are covered by an outer layer of syncytiotrophoblast and an inner layer of cytotrophoblasts, which are also known as *Langhans cells.* Cytotrophoblast proliferation at the villous tips produces the trophoblastic cell columns that form anchoring villi. They are not invaded by fetal mesenchyme, and they are anchored to the decidua at the basal plate. Thus, the base of the intervillous space faces the maternal side and consists of cytotrophoblasts from cell columns, the covering shell of syncytiotrophoblast, and maternal decidua of the basal plate. The base of the chorionic plate forms the roof of the intervillous space. It consists of two layers of trophoblasts externally and fibrous mesoderm internally. The “definitive” chorionic

plate is formed by 8 to 10 weeks as the amnionic and primary chorionic plate mesenchyme fuse together. This formation is accomplished by expansion of the amnionic sac, which also surrounds the connective stalk and the allantois and joins these structures to form the umbilical cord (Kaufmann, 1992).

Interpretation of the fine structure of the placenta came from electron microscopic studies of Wislocki and Dempsey (1955). There are prominent microvilli on the syncytial surface that correspond to the so-called brush border described by light microscopy. Associated pinocytotic vacuoles and vesicles are related to absorptive and secretory placental functions. Microvilli act to increase surface area in direct contact with maternal blood. This contact between the trophoblast and maternal blood is the defining characteristic of a hemochorial placenta (Fig. 5-10).

FIGURE 5-10

Electron micrograph of term human placenta villus. A villus capillary filled with fetal red blood cells (*asterisks*) is seen in close proximity to the microvilli border. (Reproduced with permission from Boyd JD, Hamilton WJ: The Human Placenta. Cambridge, Heffer, 1970.)



PLACENTA AND CHORION

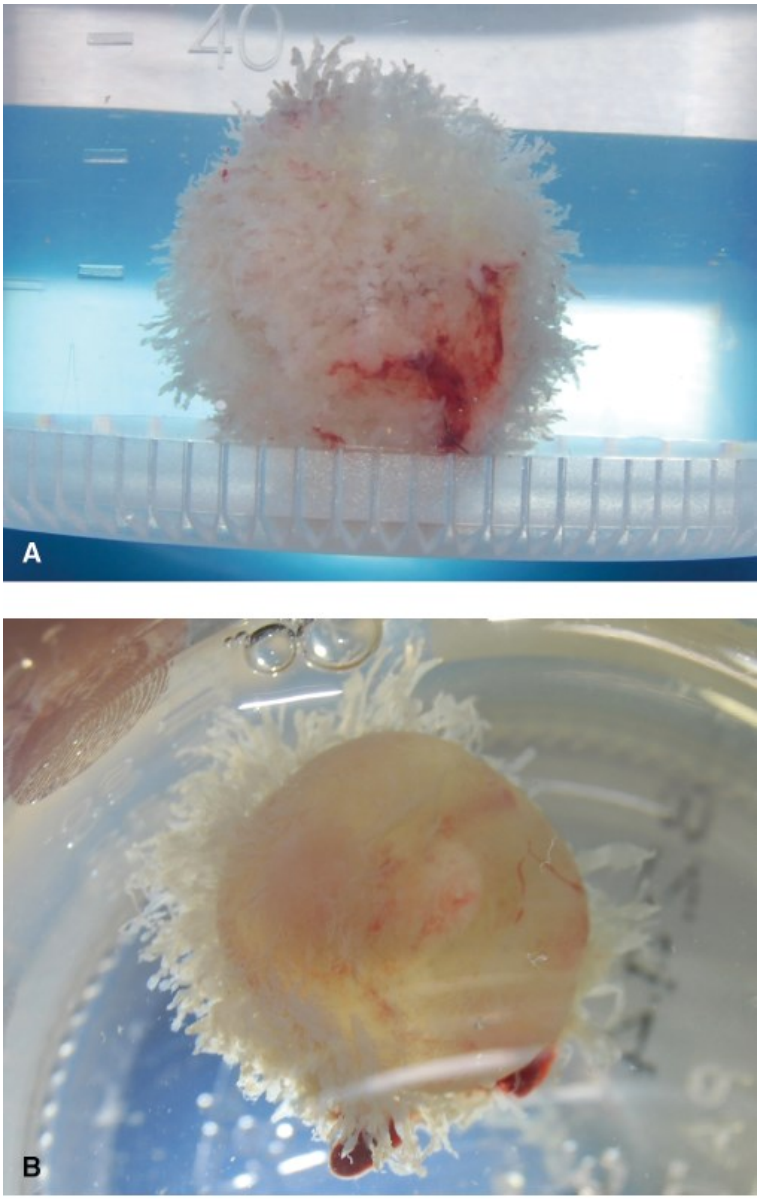
Chorion Development

In early pregnancy, the villi are distributed over the entire periphery of the chorionic membrane (Fig. 5-11). As the blastocyst with its surrounding trophoblasts grows and expands into the decidua, one pole faces the endometrial cavity. The opposite pole will form the placenta. Here, chorionic villi in contact with the decidua basalis proliferate to form the *chorion frondosum*—or leafy chorion. As growth of embryonic and extraembryonic tissues continues, the blood supply to the chorion facing the endometrial cavity is restricted. Because of this, villi in contact with the decidua capsularis cease

to grow and then degenerate. This portion of the chorion becomes the avascular fetal membrane that abuts the decidua parietalis and is called the *chorion laeve*—or smooth chorion. This smooth chorion is composed of cytotrophoblasts and fetal mesodermal mesenchyme.

FIGURE 5-11

Complete abortion specimens. **A.** Initially, the entire chorionic sac is covered with villi, and the embryo within is not visible **B.** With further growth, stretch and pressure prompt partial regression of the villi. Remaining villi form the future placenta, whereas the smooth portion is the chorion.



Until near the end of the third month, the chorion laeve is separated from the amnion by the exocoelomic cavity. Thereafter, they are in intimate contact to form an avascular amniochorion. These two structures are important sites of molecular transfer and metabolic activity. Moreover, they constitute an important paracrine arm of the fetal–maternal communication system.

Regulators of Trophoblast Invasion

Implantation and endometrial decidualization activate a unique population of maternal immune cells that infiltrate the uterus and play critical

functions in trophoblast invasion, angiogenesis, spiral artery remodeling, and maternal tolerance to fetal alloantigens. Decidual natural killer cells (dNK) make up 70 percent of decidual leukocytes in the first trimester and are found in direct contact with trophoblasts. In contrast to natural killer cells in peripheral blood, these cells lack cytotoxic functions. They produce specific cytokines and angiogenic factors to regulate invasion of fetal trophoblasts and spiral artery remodeling (Hanna, 2006). These and other unique properties distinguish dNK cells from circulating natural killer cells and from natural killer cells in the endometrium before pregnancy (Fu, 2013; Winger, 2013). dNK cells express both IL-8 and interferon-inducible protein-10, which bind to receptors on invasive trophoblastic cells to promote their decidual invasion toward the spiral arteries. dNK cells also produce proangiogenic factors, including VEGF and placental growth factor (PlGF), which both promote vascular growth in the decidua.

Trophoblasts also secrete specific chemokines that attract the dNK cells to the maternal–fetal interface. Thus, both cell types simultaneously attract each other. Decidual macrophages account for approximately 20 percent of leukocytes in the first trimester and elicit an M2-immunomodulatory phenotype (Williams, 2009). Remember, M1 macrophages are proinflammatory, and M2 macrophages counter proinflammatory responses and promote tissue repair. In addition to a role in angiogenesis and spiral artery remodeling, dNK cells promote phagocytosis of cell debris (Faas, 2017). Concurrent with the critical role of maternal dNK cells and macrophages, T cell subsets aid tolerance toward the allogenic fetus. Regulatory T cells (Tregs) are essential for promoting immune tolerance. Other T cell subsets are present, such as Th1, Th2 and Th17, although their functions are tightly regulated (Ruocco, 2014).

Endometrial Invasion

Extravillous trophoblasts of the first-trimester placenta are highly invasive. This process occurs under low-oxygen conditions, and regulatory factors that are induced under hypoxic conditions are contributory (Soares, 2012). Invasive trophoblasts secrete numerous proteolytic enzymes that digest extracellular matrix and activate proteinases already present in the decidua. Trophoblasts produce urokinase-type plasminogen activator, which converts plasminogen into the broadly acting serine protease, plasmin. This in turn both degrades matrix proteins and activates MMPs. One member of the MMP family, MMP-9, appears to be critical. The timing and extent of trophoblast invasion is regulated by a balanced interplay between pro- and antiinvasive factors.

The relative ability to invade maternal tissue in early pregnancy compared with limited invasiveness in late pregnancy is controlled by autocrine and paracrine trophoblastic and decidual factors. Trophoblasts secrete insulin-like growth factor II that promotes invasion into the decidua. Decidual cells secrete insulin-like growth factor binding-protein type 4, which blocks this autocrine loop.

Low estradiol levels in the first trimester are critical for trophoblast invasion and remodeling of the spiral arteries. Animal studies suggest that the rise in second-trimester estradiol levels suppresses and limits vessel remodeling by reducing trophoblast expression of VEGF and specific integrin receptors (Bonagura, 2012). Namely, extravillous trophoblasts express integrin receptors that recognize the extracellular matrix proteins collagen IV, laminin, and fibronectin. Binding of these matrix proteins and integrin receptors initiates signals to promote trophoblast cell migration and differentiation. However, as pregnancy advances, rising estradiol levels downregulate VEGF and integrin receptor expression. This represses and controls the extent of uterine vessel transformation.

Spiral Artery Invasion

One of the most remarkable features of human placental development is the extensive modification of maternal vasculature by trophoblasts, which are by definition of fetal origin. These events occur in the first half of pregnancy and are considered in detail because of their importance to uteroplacental blood flow. They are also integral to some pathological conditions such as preeclampsia, fetal-growth restriction, and preterm birth. Spiral artery modifications are carried out by two populations of extravillous trophoblasts—endovascular trophoblasts, which penetrate the spiral-artery lumen, and interstitial trophoblasts, which surround the arteries (see Fig. 5-8).

Interstitial trophoblasts constitute a major portion of the placental bed. They penetrate the decidua and adjacent myometrium and aggregate around spiral arteries. Although less defined, their functions may include vessel preparation for endovascular trophoblast invasion.

Endovascular trophoblasts first enter the spiral artery lumens and initially form cellular plugs. They then destroy vascular endothelium via an apoptosis mechanism and invade and modify the vascular media. Thus, fibrinoid material replaces smooth muscle and connective tissue of the vessel media. Spiral arteries later regenerate endothelium. Invading endovascular trophoblasts can extend several centimeters along the vessel lumen, and they must migrate against arterial flow. Of note, invasion by trophoblasts involves only the decidual spiral arteries and not decidual veins.

Uteroplacental vessel development proceeds in two waves or stages (Ramsey, 1980). The first occurs before 12 weeks' postfertilization, and spiral arteries are invaded and modified up to the border between the decidua and myometrium. The second wave, between 12 and 16 weeks, involves some invasion of the intramyometrial segments of spiral arteries. Remodeling converts narrow-lumen, muscular spiral arteries into dilated, low-resistance uteroplacental vessels. Molecular mechanisms of these crucial events, their regulation by cytokines, signaling pathways, and their significance in the pathogenesis of preeclampsia and fetal-growth restriction has been reviewed by several authors (Pereira de Sousa, 2017; Xie, 2016; Zhang, 2016).

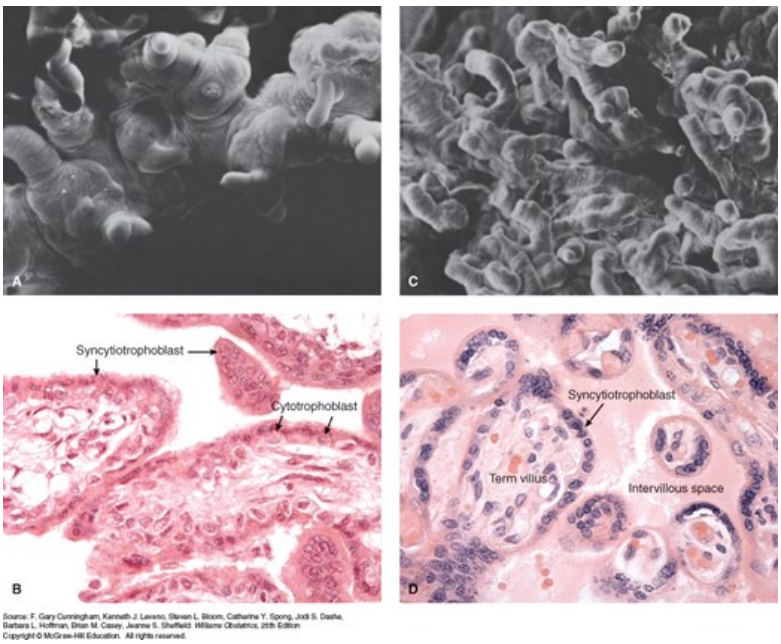
Approximately 1 month after conception, maternal blood enters the intervillous space in fountain-like bursts from the spiral arteries. Blood is propelled outside of the maternal vessels and sweeps over and directly bathes the syncytiotrophoblast.

Villus Branching

Although certain villi of the chorion frondosum extend from the chorionic plate to the decidua to serve as anchoring villi, most villi arborize and end freely within the intervillous space. As gestation proceeds, the short, thick, early stem villi branch to form progressively finer subdivisions and greater numbers of increasingly smaller villi (Fig. 5-12). Each of the truncal or main stem villi and their ramifications constitutes a placental lobule, or cotyledon. Each lobule is supplied with a single chorionic artery. And each lobule has a single vein, so that lobules constitute the functional units of placental architecture.

FIGURE 5-12

Electron micrographs **(A, C)** and photomicrographs **(B, D)** of early and late human placentas. **A** and **B.** Limited branching of villi is seen in this early placenta. **C** and **D.** With placental maturation, increasing villous arborization is seen, and villous capillaries lie closer to the surface of each villus. (Photomicrographs used with permission from Dr. Kurt Benirschke. Electron micrographs reproduced with permission from King BF, Menton DN: Scanning electron microscopy of human placental villi from early and late in gestation. Am J Obstet Gynecol 122:824, 1975.)



Placental Growth and Maturation

In the first trimester, placental growth is more rapid than that of the fetus. But by approximately 17 weeks' gestation, placental and fetal weights are approximately equal. By term, placental weight is approximately one sixth of fetal weight.

The mature placenta and its variant forms are discussed in detail in Chapter 6 (Shape and Size Variants). Briefly, viewed from the maternal surface, the number of slightly elevated convex areas, called lobes, varies from 10 to 38. Lobes are incompletely separated by grooves of variable depth that overlie placental septa, which arise as upward projections of decidua. The total number of placental lobes remains the same throughout gestation, and individual lobes continue to grow—although less actively in the final weeks (Crawford, 1959). Although grossly visible lobes are commonly referred to

as cotyledons, this is not accurate. Correctly used, lobules or cotyledons are the functional units supplied by each main stem villus.

As villi continue to branch and the terminal ramifications become more numerous and smaller, the volume and prominence of cytotrophoblasts decrease. As the syncytium thins, the fetal vessels become more prominent and lie closer to the surface (see Fig. 5-10). The villous stroma also exhibits changes as gestation progresses. In early pregnancy, the branching connective-tissue cells are separated by an abundant loose intercellular matrix. Later, the villous stroma becomes denser, and the cells are more spindly and closely packed.

Another change in the stroma involves the infiltration of *Hofbauer cells*, which are fetal macrophages. These are nearly round with vesicular, often eccentric nuclei and very granular or vacuolated cytoplasm. They grow in number and maturational state throughout pregnancy and appear to be important mediators of protection at the maternal–fetal interface (Johnson, 2012). These macrophages are phagocytic, have an immunosuppressive phenotype, can produce various cytokines, and are capable of paracrine regulation of trophoblastic functions (Cervar, 1999; Reyes, 2017). As discussed further in Chapter 64 (Coronavirus Infections), recent studies suggest that Zika virus can infect Hofbauer cells to allow fetal transmission (Simoni, 2017).

Some of the histological changes that accompany placental growth and maturation improve transport and exchange to meet advancing fetal metabolic requirements. Among these changes are a thinner syncytiotrophoblast, significantly reduced cytotrophoblast number, decreased stroma, and increased number of capillaries with close approximation to the syncytial surface. By 16 weeks' gestation, the apparent continuity of the cytotrophoblasts is lost. At term, villi may be focally reduced to a thin layer of syncytium covering minimal villous connective tissue in which thin-walled fetal capillaries abut the trophoblast and dominate the villi.

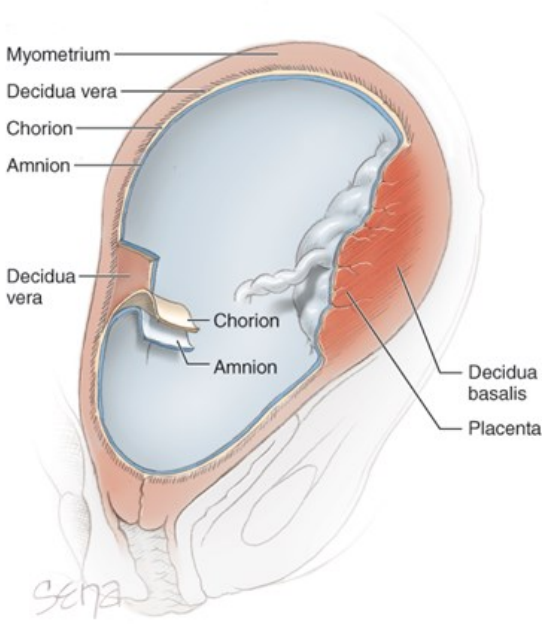
There are some changes in placental architecture that can cause decreased placental exchange efficiency if they are substantive. These include thickening of the basal lamina of trophoblast or capillaries, obliteration of certain fetal vessels, greater villous stroma, and fibrin deposition on the villous surface.

Placental Circulation

Because the placenta is functionally an intimate approximation of the fetal capillary bed to maternal blood, its gross anatomy primarily concerns vascular relations. The fetal surface is covered by the transparent amnion, beneath which chorionic vessels course. A section through the placenta includes amnion, chorion, chorionic villi and intervillous space, decidual (basal) plate, and myometrium (Figs. 5-13 and 5-14).

FIGURE 5-13

Uterus showing a normal placenta and its membranes in situ.

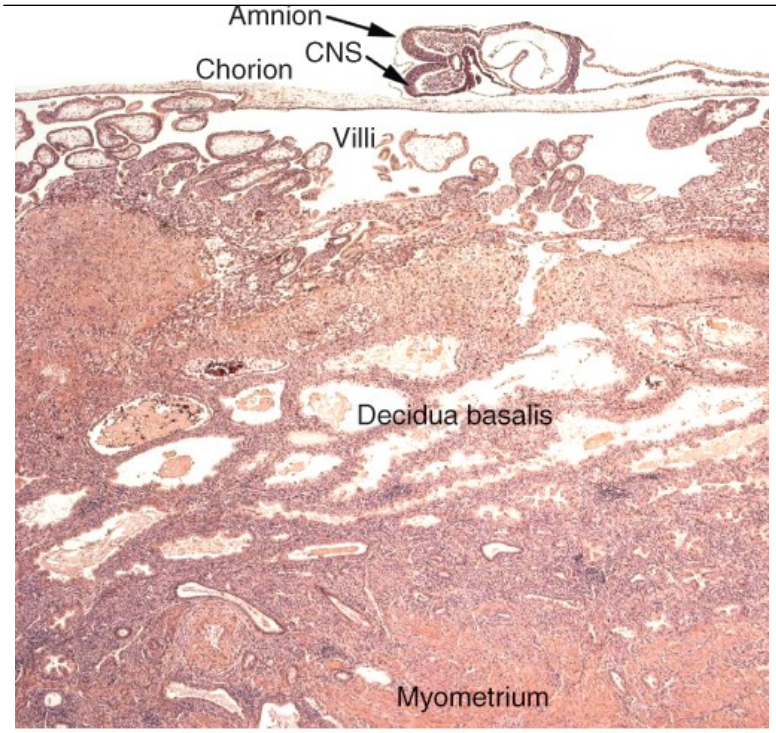


Fetal Circulation

Deoxygenated venous-like fetal blood flows to the placenta through the two umbilical arteries. As the cord joins the placenta, these umbilical vessels branch repeatedly beneath the amnion as they run across the chorionic plate. Branching continues within the villi to ultimately form capillary networks in the terminal villous branches. Blood with significantly higher oxygen content returns from the placenta via a single umbilical vein to the fetus.

FIGURE 5-14

Photomicrograph of early implanted blastocyst. Trophoblasts are seen invading the decidua basalis. CNS = central nervous system. (Used with permission from Dr. Kurt Benirschke.)



The branches of the umbilical vessels that traverse along the fetal surface of the placenta in the chorionic plate are referred to as the placental surface or chorionic vessels. These vessels are responsive to vasoactive substances, but anatomically, morphologically, histologically, and functionally, they are unique. Chorionic arteries always cross over chorionic veins. Vessels are most readily recognized by this interesting relationship, but they are difficult to distinguish by histological criteria.

Truncal arteries are perforating branches of the surface arteries that pass through the chorionic plate. Each truncal artery supplies one main stem villus and thus one cotyledon. As the artery penetrates the chorionic plate, its wall loses smooth muscle, and its caliber increases. The loss of muscle continues as the truncal arteries and veins branch into their smaller rami.

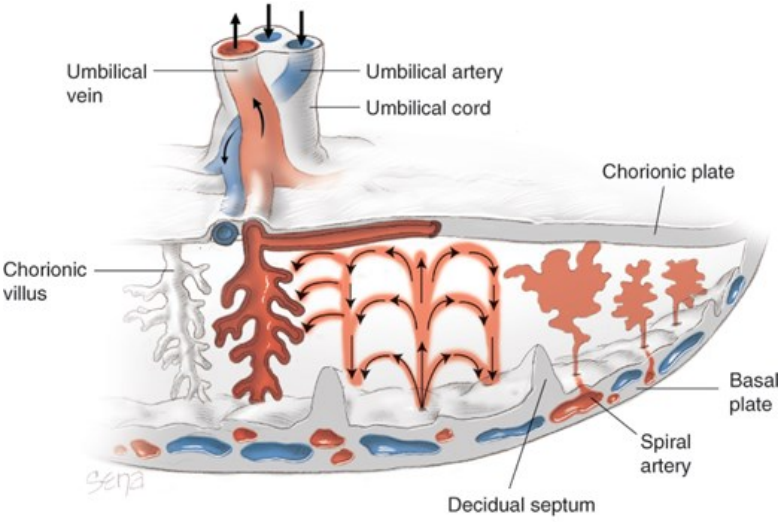
Before 10 weeks' gestation, there is no end-diastolic flow pattern within the umbilical artery at the end of the fetal cardiac cycle (Fisk, 1988; Loquet, 1988). After 10 weeks, however, end-diastolic flow appears and is maintained throughout normal pregnancy. Clinically, these flow patterns are studied with Doppler sonography to assess fetal well-being (Chap. 10, Doppler).

Maternal Circulation

Mechanisms of placental blood flow must allow blood to leave maternal circulation; flow into an amorphous space lined by syncytiotrophoblast, rather than endothelium; and return through maternal veins without producing arteriovenous-like shunts that would prevent maternal blood from remaining in contact with villi long enough for adequate exchange. For this, maternal blood enters through the basal plate and is driven high up toward the chorionic plate by arterial pressure before laterally dispersing (Fig. 5-15). After bathing the external microvillous surface of chorionic villi, maternal blood drains back through venous orifices in the basal plate and enters uterine veins. Thus, maternal blood traverses the placenta randomly without preformed channels. The previously described trophoblast invasion of the spiral arteries creates low-resistance vessels that can accommodate massive increase in uterine perfusion during gestation. Generally, spiral arteries are perpendicular to, but veins are parallel to, the uterine wall. This arrangement aids closure of veins during a uterine contraction and prevents the exit of maternal blood from the intervillous space. The number of arterial openings into the intervillous space is gradually reduced by cytotrophoblastic invasion. There are about 120 spiral arterial entries into the intervillous space at term (Brosens, 1963). These discharge blood in spurts that bathes the adjacent villi (Borell, 1958). After the 30th week, a prominent venous plexus lies between the decidua basalis and myometrium and helps develop the cleavage plane needed for placental separation after delivery.

FIGURE 5-15

Schematic drawing of a section through a full-term placenta. Maternal blood flows into the intervillous spaces in funnel-shaped spurts. Exchanges occur with fetal blood as maternal blood flows around the villi. Inflowing arterial blood pushes venous blood into the endometrial veins, which are scattered over the entire surface of the decidua basalis. Note also that the umbilical arteries carry deoxygenated fetal blood to the placenta and that the umbilical vein carries oxygenated blood to the fetus. Placental lobes are separated from each other by placental (decidual) septa.



Both inflow and outflow are curtailed during uterine contractions. Bleker and associates (1975) used serial sonography during normal labor and found that placental length, thickness, and surface area grew during contractions. They attributed this to distention of the intervillous space by impairment of venous outflow compared with arterial inflow. During contractions, therefore, a somewhat larger volume of blood is available for exchange even though the rate of flow is decreased. Similarly, Doppler velocimetry has shown that diastolic flow velocity in spiral arteries is diminished during uterine contractions. Thus, principal factors regulating intervillous space blood flow are arterial blood pressure, intrauterine pressure, uterine contraction pattern, and factors that act specifically on arterial walls.

Breaks in the Placental "Barrier"

The placenta does not maintain absolute integrity of the fetal and maternal circulations. There are numerous examples of trafficking cells between mother and fetus in both directions. This situation is best exemplified clinically by erythrocyte D-antigen alloimmunization (Chap. 15, Red Cell Alloimmunization). Fetal cell admixtures likely are small in most cases, although rarely the fetus exsanguinates into the maternal circulation.

Fetal cells can also engraft in the mother during pregnancy and can be identified decades later. Fetal lymphocytes, CD34+ mesenchymal stem cells, and endothelial colony-forming cells all reside in maternal blood, bone marrow, or uterine vasculature (Nguyen, 2006; Piper, 2007; Sipos, 2013). Termed *microchimerism*, such residual stem cells have been implicated in the disparate female:male ratio of autoimmune disorders (Greer, 2011; Stevens, 2006). As discussed in Chapter 59 (Systemic Lupus Erythematosus), they are associated with the pathogenesis of lymphocytic thyroiditis, scleroderma, and systemic lupus erythematosus.

Fetal–Maternal Interface

Survival of the semiallogenic fetal graft requires complex interactions between fetal trophoblasts and maternal decidual immune cells. The fetal–maternal interface is not immunologically inert. Rather, it is an active hub of interactions that allows implantation and appropriate placental development and ensures immunotolerance of the fetus. Despite this, a functional immune system must be maintained to protect the mother.

Immunogenicity of the Trophoblasts

Trophoblastic cells are the only fetus-derived cells in direct contact with maternal tissues and blood. Fetal syncytiotrophoblast synthesizes and

secretes numerous factors that regulate the immune responses of maternal cells both at the implantation site and systemically.

Human leukocyte antigens (HLAs) are the human analogue of the major histocompatibility complex (MHC) (Hunt, 1992). There are 17 HLA class I genes, including three classic genes, HLA-A, -B, and -C, that encode the major class I (class Ia) transplantation antigens. Three other class I genes, designated HLA-E, -F, and -G, encode class Ib HLA antigens. MHC class I and II antigens are absent from villous trophoblasts, which appear to be immunologically inert at all gestational stages (Weetman, 1999). Invasive extravillous cytotrophoblasts do express MHC class I molecules. Thus, the ability of these cells to bypass transplantation rejection is the focus of considerable study.

Moffett-King (2002) reasoned that normal implantation depends on controlled trophoblastic invasion of maternal decidua and spiral arteries. Such invasion must proceed far enough to provide for normal fetal growth and development, but a mechanism must regulate invasion depth. She suggests that dNK cells combined with unique expression of three specific HLA class I genes in extravillous cytotrophoblasts act in concert to permit and subsequently limit trophoblast invasion.

Class I antigens in extravillous cytotrophoblasts are accounted for by the expression of classic HLA-C and nonclassic class Ib molecules of HLA-E and HLA-G. HLA-G antigen is expressed only in humans, with expression restricted to extravillous cytotrophoblasts contiguous with maternal tissues. Embryos used for in vitro fertilization do not implant if they do not express a soluble HLA-G isoform (Fuzzi, 2002). Thus, HLA-G may be immunologically permissive of the maternal–fetal antigen mismatch (LeBouteiller, 1999). HLA-G has a proposed role in protecting extravillous trophoblasts from immune rejection via modulation of dNK functions (Apps, 2011; Rajagopalan, 2012). Last, Goldman-Wohl and associates (2000) have provided evidence for abnormal HLA-G expression in extravillous trophoblasts from women with preeclampsia.

Decidual Immune Cells

Natural killer cells are the predominant population of leukocytes present in midluteal phase endometrium and in decidua throughout the first trimester (Johnson, 1999). By term, however, relatively few dNK cells are present in decidua. In first-trimester decidua, dNK cells lie close to extravillous trophoblasts, and there they purportedly serve to regulate invasion. These dNKs have a distinct phenotype characterized by a high surface density of CD56 or neural cell adhesion molecules (Manaster, 2008; Moffett-King, 2002). Their infiltration is increased by progesterone and by stromal cell production of IL-15 and decidual prolactin (Dunn, 2002; Gubbay, 2002). Although dNK cells have the capacity for cytotoxicity, they are not cytotoxic toward fetal trophoblasts. Their cytotoxic potential is prevented by molecular cues from decidual macrophages. In addition, the expression of specific HLA molecules protects against dNK killing. Also, dNK cells function to restrict trophoblast invasiveness to protect the mother.

Of other cell types, *decidual macrophages* are distinct from proinflammatory M1 or antiinflammatory M2 macrophages**.** Decidual macrophages express the complement receptor CD11c at high or low levels: CD11cHI and CD11cLO. These cells function to regulate adaptive T cell responses; control dNK differentiation, activation, and cytotoxicity; and produce antiinflammatory cytokines such as IL-10 to ensure fetal tolerance and inhibition of harmful immune responses.

Dendritic cells are cells that present antigens to T cells. They play an important role in the development of a receptive endometrium for implantation.

Maternal T cells, as part of the adaptive immune response, increase in number and function after encounter with a specific antigen. These cells subsequently retain the ability to respond rapidly in a subsequent encounter with the same antigen. Specific populations of Treg cells persist and can protect against aberrant immune responses. During pregnancy, there is a systemic expansion of maternal Treg cell populations. These cells are FOXP3+ cells with defined fetal specificity. They are immunosuppressive and play a role in fetal tolerance.

AMNION

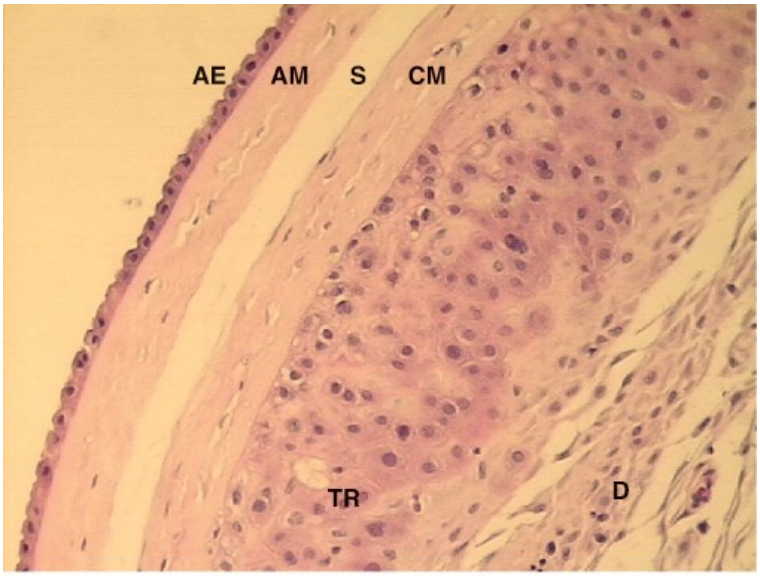
At term, the amnion is a tough and tenacious but pliable membrane. This innermost avascular fetal membrane is contiguous with amnionic fluid and plays a role of incredible importance in human pregnancy. The amnion provides almost all tensile strength of the fetal membranes. Thus, its resilience to rupture is vitally important to successful pregnancy outcome. Indeed, preterm rupture of fetal membranes is a major cause of preterm delivery (Chap. 42, Management of Preterm Premature Rupture of Membranes).

Bourne (1962) described five separate amnion layers. The inner surface, which is bathed by amnionic fluid, is an uninterrupted, single layer of cuboidal epithelium (Fig. 5-16). This epithelium is attached firmly to a distinct basement membrane that is connected to an acellular compact layer composed primarily of interstitial collagens. On the outer side of the compact layer, there is a row of fibroblast-like mesenchymal cells, which are widely dispersed

at term. There also are a few fetal macrophages in the amnion. The outermost amnion layer is the relatively acellular zona spongiosa, which is contiguous with the second fetal membrane, the chorion laeve. The human amnion lacks smooth muscle cells, nerves, lymphatics, and importantly, blood vessels.

FIGURE 5-16

Photomicrograph of fetal membranes. From left to right: AE = amnion epithelium; AM = amnion mesenchyme; S = zona spongiosa; CM = chorionic mesenchyme; TR = trophoblast; D = decidua. (Used with permission from Dr. Judith R. Head.)



Amnion Development

Early during implantation, a space develops between the embryonic cell mass and adjacent trophoblastic cells (see Fig. 5-9). Small cells that line this inner surface of trophoblasts have been called amniogenic cells—precursors of amnionic epithelium. The amnion is first identifiable on the 7th or 8th day of embryo development. It is initially a minute vesicle, which then develops into a small sac that covers the dorsal embryo surface. As the amnion enlarges, it gradually engulfs the growing embryo, which prolapses into its cavity (Benirschke, 2012).

Distention of the amnionic sac eventually brings it into contact with the interior surface of the chorion laeve. Apposition of the chorion laeve and amnion near the end of the first trimester then causes an obliteration of the extraembryonic coelom. The amnion and chorion laeve, although slightly adhered, are never intimately connected and can be separated easily. Placental amnion covers the placental surface and thereby is in contact with the adventitial surface of chorionic vessels. Umbilical amnion covers the umbilical cord. With diamnionic-monochorionic placentas, there is no intervening tissue between the fused amnions. In the conjoined portion of membranes of diamnionic-dichorionic twin placentas, amnions are separated by fused chorion laeve.

Amnionic fluid fills this amnionic sac. Until about 34 weeks' gestation, the normally clear fluid increases in volume as pregnancy progresses. After this, the volume declines. At term, amnionic fluid averages 1000 mL, although this may vary widely in normal and especially abnormal conditions. The origin, composition, circulation, and function of amnionic fluid are discussed further in Chapter 11 (Normal Amnionic Fluid Volume).

Amnion Cell Histogenesis

Epithelial cells of the amnion derive from fetal ectoderm of the embryonic disc. They do not arise by delamination from trophoblasts. This is an important consideration from both embryological and functional perspectives. For example, HLA class I gene expression in amnion is more akin to that in embryonic cells than to that in trophoblasts.

The fibroblast-like mesenchymal cell layer of the amnion is likely derived from embryonic mesoderm. Early in human embryogenesis, the amnionic mesenchymal cells lie immediately adjacent to the basal surface of the amnion epithelium. At this time, the amnion surface is a two-cell layer with approximately equal numbers of epithelial and mesenchymal cells. Simultaneously with growth and development, interstitial collagens are deposited between these two cell layers. This marks formation of the amnion compact layer, which separates the two layers of amnion cells.

As the amnionic sac expands, the compactness of the mesenchymal cells is progressively reduced, and they become sparsely distributed. Early in pregnancy, amnionic epithelium replicates at a rate appreciably faster than mesenchymal cells. At term, these cells form a continuous uninterrupted epithelium on the fetal amnionic surface. Conversely, mesenchymal cells are widely dispersed, being connected by a fine lattice network of extracellular matrix with the appearance of long slender fibrils.

Amnion Epithelial Cells

The apical surface of the amnionic epithelium is replete with highly developed microvilli. This structure reflects its function as a major site of transfer between amnionic fluid and amnion. This epithelium is metabolically active, and its cells synthesize tissue inhibitor of MMP-1, prostaglandin E_2 (PGE_2), and fetal fibronectin (fFN) (Rowe, 1997). Although epithelia produce fFN, recent studies suggest that fibronectin functions in the underlying mesenchymal cells. Here, fFN promotes synthesis of MMPs that break down the strength-bearing collagens and enhance prostaglandin synthesis to prompt uterine contractions (Mogami, 2013). This pathway is upregulated in premature rupture of membranes induced by thrombin or infection-induced release of fFN (Chigusa, 2016; Mogami, 2014).

Epithelial cells may respond to signals derived from the fetus or the mother, and they are responsive to various endocrine or paracrine modulators. Examples include oxytocin and vasopressin, both of which increase PGE_2 production in vitro (Moore, 1988). They may also produce cytokines such as IL-8 during labor initiation (Elliott, 2001).

Amnionic epithelium also synthesizes vasoactive peptides, including endothelin and parathyroid hormone-related protein (Economos, 1992; Germain, 1992). The tissue produces brain natriuretic peptide (BNP) and corticotropin-releasing hormone (CRH), which are peptides that invoke smooth-muscle relaxation (Riley, 1991; Warren, 1995). BNP production is positively regulated by mechanical stretch in fetal membranes and is proposed to function in uterine quiescence. Epidermal growth factor, a negative regulator of BNP, is upregulated in the membranes at term and leads to a decline in BNP-regulated uterine quiescence (Carvajal, 2013). It seems reasonable that vasoactive peptides produced in amnion gain access to the adventitial surface of chorionic vessels. Thus, the amnion may be involved in modulating chorionic vessel tone and blood flow. Amnion-derived vasoactive peptides function in both maternal and fetal tissues in diverse physiological processes. After their secretion, these bioactive agents enter amnionic fluid and thereby are available to the fetus by swallowing and inhalation.

Amnion Mesenchymal Cells

Mesenchymal cells of the amnionic fibroblast layer are responsible for other major functions. Synthesis of interstitial collagens that compose the compact layer of the amnion—the major source of its tensile strength—takes place in mesenchymal cells (Casey, 1996). At term the generation of cortisol by 11β-hydroxysteroid dehydrogenase may contribute to membrane rupture via reduction of collagen abundance (Mi, 2017). Mesenchymal cells also synthesize cytokines that include IL-6, IL-8, and MCP-1. Cytokine synthesis rises in response to bacterial toxins and IL-1. This functional capacity of amnion mesenchymal cells is an important consideration in amnionic fluid study of labor-associated accumulation of inflammatory mediators (Garcia-Velasco, 1999). Finally, mesenchymal cells may be a greater source of PGE_2 than epithelial cells, especially in the case of premature membrane rupture (Mogami, 2013; Whittle, 2000).

Tensile Strength

During tests of tensile strength, the decidua and then the chorion laeve give way long before the amnion ruptures. Indeed, the membranes are elastic and can expand to twice normal size during pregnancy (Benirschke, 2012). The amnion tensile strength resides almost exclusively in the compact layer, which is composed of cross-linked interstitial collagens I and III and lesser amounts of collagens V and VI.

Collagens are the primary macromolecules of most connective tissues. Collagen I is the major interstitial collagen in tissues characterized by great tensile strength, such as bone and tendon. In other tissues, collagen III is believed to contribute to tissue integrity and provides both tissue extensibility and tensile strength. For example, the ratio of collagen III to collagen I in the walls of a number of highly extensible tissues—amnionic sac, blood

vessels, urinary bladder, bile ducts, intestine, and gravid uterus—is greater than that in nonelastic tissues (Jeffrey, 1991).

Amnion tensile strength is regulated in part by fibrillar collagen assembly. This process is influenced by the interaction fibrils with proteoglycans such as decorin and biglycan (Chap. 21, Cervical Ripening). Reduction of these proteoglycans is reported to perturb fetal membrane function (Horgan, 2014; Wu, 2014). Fetal membranes overlying the cervix have a regional shift in gene expression and lymphocyte activation that set in motion an inflammatory cascade (Marcellin, 2017). This change may contribute to tissue remodeling and loss of tensile strength in the amnion (Moore, 2009).

Metabolic Functions

The amnion is metabolically active, is involved in solute and water transport for amnionic fluid homeostasis, and produces an impressive array of bioactive compounds. The amnion is responsive both acutely and chronically to mechanical stretch, which alters amnionic gene expression (Carvajal, 2013; Nemeth, 2000). This in turn may trigger both autocrine and paracrine responses that include production of MMPs, IL-8, and collagenase (Bryant-Greenwood, 1998; Mogami, 2013). Such factors may modulate changes in membrane properties during labor.

UMBILICAL CORD

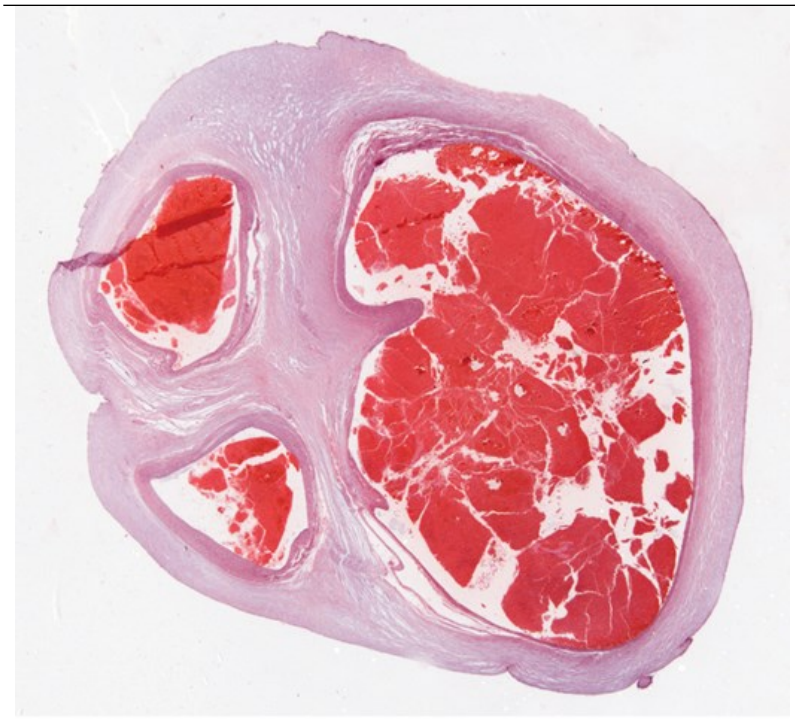
The yolk sac and the umbilical vesicle into which it develops are prominent early in pregnancy. At first, the embryo is a flattened disc interposed between amnion and yolk sac (see Fig. 5-9). Its dorsal surface grows faster than the ventral surface, in association with the elongation of its neural tube. Thus, the embryo bulges into the amnionic sac, and the dorsal part of the yolk sac is incorporated into the embryo body to form the gut. The allantois projects into the base of the body stalk from the caudal wall of the yolk sac and later, from the anterior wall of the hindgut.

As pregnancy advances, the yolk sac becomes smaller and its pedicle relatively longer. By the middle of the third month, the expanding amnion obliterates the extraembryonic coelom, fuses with the chorion laeve, and covers the bulging placental disc and the lateral surface of the body stalk. The latter is then called the umbilical cord—or funis. A more detailed description of this cord and potential abnormalities is found in Chapter 6 (Umbilical Cord).

The cord at term normally has two arteries and one vein (Fig. 5-17). The right umbilical vein usually disappears early during fetal development, leaving only the original left vein.

FIGURE 5-17

Cross-section of umbilical cord. The large umbilical vein carries oxygenated blood to the fetus *(right)*. To its left are the two smaller umbilical arteries, carrying deoxygenated blood from the fetus to the placenta. (Used with permission from Dr. Mandolin S. Ziadie.)



The umbilical cord extends from the fetal umbilicus to the fetal surface of the placenta, that is, the chorionic plate. Blood flows from the umbilical vein toward the fetus. Blood then takes a path of least resistance via two routes within the fetus. One is the ductus venosus, which empties directly into the inferior vena cava (Fig. 7-9). The other route consists of numerous smaller openings into the hepatic circulation. Blood from the liver flows into the inferior vena cava via the hepatic vein. Resistance in the ductus venosus is controlled by a sphincter that is situated at the origin of the ductus at the umbilical recess and is innervated by a vagus nerve branch.

Blood exits the fetus via the two umbilical arteries. These are anterior branches of the internal iliac artery and become obliterated after birth to form the medial umbilical ligaments.

PLACENTAL HORMONES

The production of steroid and protein hormones by human trophoblasts is greater in amount and diversity than that of any single endocrine tissue in all of mammalian physiology. A compendium of average production rates for various steroid hormones in nonpregnant and in near-term pregnant women is given in Table 5-1. It is apparent that alterations in steroid hormone production that accompany normal human pregnancy are incredible. The human placenta also synthesizes an enormous amount of protein and peptide hormones, summarized in Table 5-2. Another remarkable feature is the successful physiological adaptations of pregnant women to the unique endocrine milieu, and this is discussed throughout Chapter 4 (Reproductive Tract).

TABLE 5-1

Steroid Production Rates in Nonpregnant and Near-Term Pregnant Women

	Production Rates (mg/24 hr)	
Steroid[a]	**Nonpregnant**	**Pregnant**
Estradiol-17β	0.1–0.6	15–20
Estriol	0.02–0.1	50–150
Progesterone	0.1–40	250–600
Aldosterone	0.05–0.1	0.250–0.600
Deoxycorticosterone	0.05–0.5	1–12
Cortisol	10–30	10–20

[a]Estrogens and progesterone are produced by placenta. Aldosterone is produced by the maternal adrenal in response to the stimulus of angiotensin II. Deoxycorticosterone is produced in extraglandular tissue sites by way of the 21-hydroxylation of plasma progesterone. Cortisol production during pregnancy is not increased, even though the blood levels are elevated because of decreased clearance caused by increased cortisol-binding globulin.

TABLE 5-2

Protein Hormones Produced by the Human Placenta

Hormone	Primary Non-placental Site of Expression	Shares Structural or Function Similarity	Functions
Human chorionic gonadotropin (hCG)	—	LH, FSH, TSH	Maintains corpus luteum function Regulates fetal testis testosterone secretion Stimulates maternal thyroid
Placental lactogen (PL)	—	GH, prolactin	Aids maternal adaptation to fetal energy requirements
Adrenocorticotropin (ACTH)	Hypothalamus	—	
Corticotropin-releasing hormone (CRH)	Hypothalamus	—	Relaxes smooth-muscle; initiates parturition? Promotes fetal and maternal glucocorticoid production
Gonadotropin-releasing hormone (GnRH)	Hypothalamus	—	Regulates trophoblast hCG production
Thyrotropin (TRH)	Hypothalamus		Unknown
Growth hormone- releasing hormone (GHRH)	Hypothalamus	—	Unknown
Growth hormone variant (hGH-V)	—	GH variant not found in pituitary	Potentially mediates pregnancy insulin resistance
Neuropeptide Y	Brain		Potential regulates CRH release by trophoblasts
Parathyroid-releasing protein (PTH-rp)	—		Regulates transfer of calcium and other solutes; regulates fetal mineral homeostasis
Inhibin	Ovary/testis		Potentially inhibits FSH-mediated ovulation; regulates hCG synthesis
Activin	Ovary/testis		Regulates placental GnRH synthesis

GH = growth hormone; FSH = follicle-stimulating hormone; LH = luteinizing hormone; TSH = thyroid-stimulating hormone.

Human Chorionic Gonadotropin

Biosynthesis

Chorionic gonadotropin is a glycoprotein with biological activity similar to that of LH, and both act via the same LH-hCG receptor. hCG has with a molecular weight of 36,000 to 40,000 Da and has the highest carbohydrate content of any human hormone—30 percent. The carbohydrate component, and especially the terminal sialic acid, protects the molecule from catabolism. As a result, the 36-hour plasma half-life of intact hCG is much longer than the 2 hours for LH. The hCG molecule is composed of two dissimilar subunits termed α and β subunits. These are noncovalently linked and are held

together by electrostatic and hydrophobic forces. Isolated subunits are unable to bind the LH-hCG receptor and thus lack biological activity.

hCG is produced almost exclusively in the placenta, but low levels are synthesized in the fetal kidney. Other fetal tissues produce either the β-subunit or intact hCG molecule (McGregor, 1981, 1983).

The hCG hormone is structurally related to three other glycoprotein hormones—LH, FSH, and TSH. All four glycoproteins share a common α-subunit. However, each of their β-subunits, although sharing certain similarities, is characterized by a distinctly different amino acid sequence.

Synthesis of the α- and β-chains of hCG is regulated separately. A single gene located on chromosome 6 encodes the α-subunit. Seven genes on chromosome 19 encode for the β-hCG–β-LH family of subunits. Six genes code for β-hCG and one for β-LH (Miller-Lindholm, 1997). Both subunits are synthesized as larger precursors, which are then cleaved by endopeptidases. Intact hCG is then assembled and rapidly released by secretory granule exocytosis (Morrish, 1987). There are multiple forms of hCG in maternal plasma and urine that vary enormously in bioactivity and immunoreactivity. Some result from enzymatic degradation, and others from modifications during molecular synthesis and processing.

Before 5 weeks, hCG is expressed both in the syncytiotrophoblast and in cytotrophoblasts (Maruo, 1992). Later, in the first trimester when maternal serum levels peak, hCG is produced almost solely in the syncytiotrophoblast (Beck, 1986; Kurman, 1984). At this time, mRNA concentrations of both α- and β-subunits in the syncytiotrophoblast are greater than at term (Hoshina, 1982). This may be an important consideration when hCG is used as a screening procedure to identify abnormal fetuses.

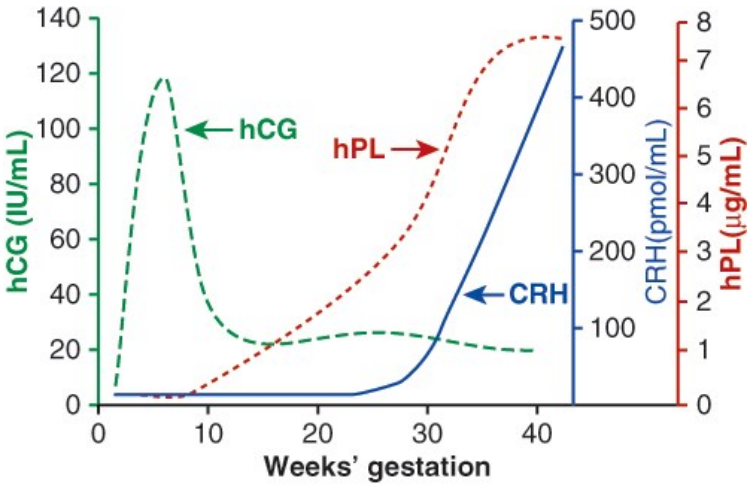
Circulating free levels of β-subunit are low to undetectable throughout pregnancy. In part, this is the result of its rate-limiting synthesis. Free α-subunits that do not combine with the β-subunit are found in placental tissue and maternal plasma. Levels of the α-subunit rise gradually and steadily until they plateau at approximately 36 weeks' gestation. At this time, they account for 30 to 50 percent of hormone (Cole, 1997). Thus, α-hCG secretion roughly corresponds to placental mass, whereas secretion of complete hCG molecules is maximal at 8 to 10 weeks.

Concentrations in Serum and Urine

The combined hCG molecule is detectable in plasma of pregnant women 7 to 9 days after the midcycle surge of LH that precedes ovulation. Thus, hCG likely enters maternal blood at the time of blastocyst implantation. Plasma levels rise rapidly, doubling every 2 days in the first trimester (Fig. 5-18). Appreciable fluctuations in levels for a given patient are observed on the same day.

FIGURE 5-18

Distinct profiles for the concentrations of human chorionic gonadotropin (hCG), human placental lactogen (hPL), and corticotropin-releasing hormone (CRH) in serum of women throughout normal pregnancy.



Source: F. Gary Cunningham, Kenneth J. Leveno, Steven L. Bloom, Catherine Y. Spong, Jodi S. Dashe, Barbara L. Hoffman, Brian M. Casey, Jeanne S. Sheffield: *Williams Obstetrics*, 25th Edition

Intact hCG circulates as multiple highly related isoforms with variable cross-reactivity between commercial assays. Thus, calculated serum hCG levels can vary considerably among the more than a hundred available assays. This emphasizes the need to use the same assay type when clinically

measuring serial hCG levels. Peak maternal plasma levels reach approximately 50,000 to 100,000 mIU/mL between the 60th and 80th days after menses. At 10 to 12 weeks' gestation, plasma levels begin to decline, and a nadir is reached by approximately 16 weeks. Plasma levels are maintained at this lower level for the remainder of pregnancy.

The pattern of hCG appearance in fetal blood is similar to that in the mother. Fetal plasma levels, however, are only about 3 percent of those in maternal plasma. Amnionic fluid hCG concentration early in pregnancy is similar to that in maternal plasma. As pregnancy progresses, hCG concentration in amnionic fluid declines, and near term the levels are approximately 20 percent of those in maternal plasma.

Maternal urine contains the same variety of hCG degradation products as maternal plasma. The principal urinary form is the terminal product of hCG degradation, namely, the β-core fragment. Concentrations of this fragment follow the same general pattern as that in maternal plasma, peaking at approximately 10 weeks' gestation. Importantly, the so-called β-subunit antibody used in most pregnancy tests reacts with both intact hCG—the major form in the plasma, and with fragments of hCG—the major forms found in urine.

hCG Regulation

Placental gonadotropin-releasing hormone (GnRH) is likely involved in the regulation of hCG formation. Both GnRH and its receptor are expressed by cytotrophoblasts and by the syncytiotrophoblast (Wolfahrt, 1998). GnRH administration elevates circulating hCG levels, and cultured trophoblasts respond to GnRH treatment and raise hCG secretion (Iwashita, 1993; Siler-Khodr, 1981). Pituitary GnRH production also is regulated by inhibin and activin. In cultured placental cells, activin stimulates and inhibin inhibits GnRH and hCG production (Petraglia, 1989; Steele, 1993).

Renal clearance of hCG accounts for 30 percent of its metabolic clearance. The remainder is likely cleared by metabolism in the liver (Wehmann, 1980). Clearances of β- and α-subunits are approximately 10- and 30-fold, respectively, greater than that of intact hCG. In pregnancies complicated by chronic renal disease, hCG clearance can be markedly decreased.

Biological Functions

Both hCG subunits are required for binding to the LH-hCG receptor in the corpus luteum and the fetal testis. LH-hCG receptors are present in various other tissues, but their roles there are less defined.

The best-known biological function of hCG is the so-called rescue and maintenance of corpus luteum function—that is, continued progesterone production. This is only an incomplete explanation for the physiological function of hCG in pregnancy. For example, maximum plasma hCG concentrations are attained well after hCG-stimulated corpus luteum secretion of progesterone has ceased. Specifically, luteal progesterone synthesis begins to decline at about 6 weeks despite continued and increasing hCG production.

A second hCG role is stimulation of fetal testicular testosterone secretion. This is maximum approximately when hCG levels peak. Thus, at a critical time in male sexual differentiation, hCG enters fetal plasma from the syncytiotrophoblast. In the fetus, it acts as an LH surrogate to stimulate Leydig cell replication and testosterone synthesis to promote male sexual differentiation (Chap. 3, Embryology of the Gonads). Before approximately 110 days, there is no vascularization of the fetal anterior pituitary from the hypothalamus. Thus, pituitary LH secretion is minimal, and hCG acts as LH before this time. Thereafter, as hCG levels fall, pituitary LH maintains modest testicular stimulation.

The maternal thyroid gland is also stimulated by large quantities of hCG. In some women with gestational trophoblastic disease, biochemical and clinical evidence of hyperthyroidism sometimes develops (Chap. 20, Diagnosis). This once was attributed to formation of chorionic thyrotropins by neoplastic trophoblasts. It was subsequently shown, however, that some forms of hCG bind to TSH receptors on thyrocytes (Hershman, 1999). And, treatment of men with exogenous hCG increases thyroid activity. The thyroid-stimulatory activity in plasma of first-trimester pregnant women varies appreciably from sample to sample. Modifications of hCG oligosaccharides likely are important in the capacity of hCG to stimulate thyroid function. For example, acidic isoforms stimulate thyroid activity, and some more basic isoforms stimulate iodine uptake (Kraiem, 1994; Tsuruta, 1995; Yoshimura, 1994). Finally, the LH-hCG receptor is expressed by thyrocytes, which suggests that hCG stimulates thyroid activity via the LH-hCG receptor as well as by the TSH receptor (Tomer, 1992).

Other hCG functions include promotion of relaxin secretion by the corpus luteum (Duffy, 1996). LH-hCG receptors are found in myometrium and in uterine vascular tissue. It has been hypothesized that hCG may promote uterine vascular vasodilatation and myometrial smooth muscle relaxation (Kurtzman, 2001). Chorionic gonadotropin also regulates expansion of dNK cell numbers during early stages of placentation, thus ensuring

appropriate establishment of pregnancy (Kane, 2009).

Abnormally High or Low Levels

There are several clinical circumstances in which substantively higher maternal plasma hCG levels are found. Some examples are multifetal pregnancy, erythroblastosis fetalis associated with fetal hemolytic anemia, and gestational trophoblastic disease. Relatively higher hCG levels may be found in women carrying a fetus with Down syndrome. This observation is used in biochemical screening tests (Chap. 14, Traditional Aneuploidy Screening Tests). The reason for the elevation is not clear, but reduced placental maturity has been speculated. Various malignant tumors also produce hCG, sometimes in large amounts—especially trophoblastic neoplasms (Chaps. 9 (Initial Prenatal Evaluation) and 20, Diagnosis).

Relatively lower hCG plasma levels are found in women with early pregnancy wastage, including ectopic pregnancy (Chap. 19, Multimodality Diagnosis). hCG is produced in very small amounts in normal tissues of men and nonpregnant women, perhaps primarily in the anterior pituitary gland. Nonetheless, the detection of hCG in blood or urine almost always indicates pregnancy (Chap. 9, Diagnosis of Pregnancy).

Human Placental Lactogen

Biosynthesis

This is a single, nonglycosylated polypeptide chain with a molecular weight of 22,279 Da. The sequences of hPL and of human growth hormone (hGH) are strikingly similar, with 96-percent homology. Also, hPL is structurally similar to human prolactin (hPRL), with a 67-percent amino acid sequence similarity. Because of these similarities, it was called human placental lactogen or chorionic growth hormone. Currently, human placental lactogen is used by most.

There are five genes in the growth hormone–placental lactogen gene cluster that are linked and located on chromosome 17. hPL is concentrated in syncytiotrophoblast, but similar to hCG, hPL is demonstrated in cytotrophoblasts before 6 weeks (Grumbach, 1964; Maruo, 1992). Within 5 to 10 days after conception, hPL is demonstrable in the placenta and can be detected in maternal serum as early as 3 weeks. Levels of mRNA for hPL in syncytiotrophoblast remain relatively constant throughout pregnancy. This finding supports the idea that the hPL secretion rate is proportional to placental mass. Levels rise steadily until 34 to 36 weeks' gestation. The hPL production rate near term—approximately 1 g/d—is by far the greatest of any known hormone in humans. The half-life of hPL in maternal plasma is between 10 and 30 minutes (Walker, 1991). In late pregnancy, maternal serum concentrations reach levels of 5 to 15 µg/mL (see Fig. 5-18).

Very little hPL is detected in fetal blood or in the urine of the mother or newborn. Amnionic fluid levels are somewhat lower than in maternal plasma. hPL is secreted primarily into the maternal circulation, with only very small amounts in cord blood. Thus, its role in pregnancy is believed to be mediated through actions in maternal rather than in fetal tissues. Nonetheless, interest continues for the possibility that hPL serves select functions in fetal growth.

Metabolic Actions

hPL has putative actions in several important metabolic processes. First, hPL promotes maternal lipolysis with increased circulating free fatty acid levels. This provides an energy source for maternal metabolism and fetal nutrition. In vitro studies suggest that hPL inhibits leptin secretion by term syncytiotrophoblast (Coya, 2005). Prolonged maternal starvation in the first half of pregnancy leads to higher hPL plasma concentrations.

Second, hPL may aid maternal adaptation to fetal energy requirements. For example, increased maternal insulin resistance ensures nutrient flow to the fetus. It also favors protein synthesis and provides a readily available amino acid source to the fetus. To counterbalance the greater insulin resistance and prevent maternal hyperglycemia, maternal insulin levels are increased. Both hPL and prolactin signal through the prolactin receptor to increase maternal beta cell proliferation to augment insulin secretion (Georgia, 2010). In animals, prolactin and hPL upregulate serotonin synthesis, which increases beta cell proliferation (Kim, 2010). Short-term changes in plasma glucose or insulin, however, have relatively little effect on plasma hPL levels. In vitro studies of syncytiotrophoblast suggest that hPL synthesis is stimulated by insulin and insulin-like growth factor-1 and inhibited by PGE_2 and $PGF_{2\alpha}$ (Bhaumick, 1987; Genbacev, 1977).

Last, hPL is a potent angiogenic hormone. It may serve an important function in fetal vasculature formation (Corbacho, 2002).

Other Placental Protein Hormones

The placenta has a remarkable capacity to synthesize numerous peptide hormones, including some that are analogous or related to hypothalamic and pituitary hormones. In contrast to their counterparts, some of these placental peptide/protein hormones are not subject to feedback inhibition.

Hypothalamic-Like Releasing Hormones

The known hypothalamic-releasing or -inhibiting hormones include GnRH, CRH, thyrotropin-releasing hormone (TRH), growth hormone-releasing hormone, and somatostatin. For each, there is an analogous hormone produced in the human placenta (Petraglia, 1992; Siler-Khodr, 1988).

GnRH in the placenta shows its highest expression in the first trimester (Siler-Khodr, 1978, 1988). Interestingly, it is found in cytotrophoblasts, but not syncytiotrophoblast. Placenta-derived GnRH functions to regulate trophoblast hCG production and extravillous trophoblast invasion via regulation of MMP-2 and MMP-9 (Peng, 2016). Placenta-derived GnRH is also the likely cause of elevated maternal GnRH levels in pregnancy (Siler-Khodr, 1984).

CRH is a member of a larger family of CRH-related peptides that includes CRH and urocortins (Dautzenberg, 2002). Maternal serum CRH levels increase from 5 to 10 pmol/L in the nonpregnant woman to approximately 100 pmol/L in the early third trimester of pregnancy and to almost 500 pmol/L abruptly during the last 5 to 6 weeks (see Fig. 5-18). Urocortin also is produced by the placenta and secreted into the maternal circulation, but at much lower levels than seen for CRH (Florio, 2002). After labor begins, maternal plasma CRH levels rise even further (Petraglia, 1989, 1990).

The biological function of CRH synthesized in the placenta, membranes, and decidua has been somewhat defined. CRH receptors are present in many tissues including placenta. Trophoblast, amniochorion, and decidua express both CRH-R1 and CRH-R2 receptors and several variant receptors (Florio, 2000). Both CRH and urocortin enhance trophoblast secretion of adrenocorticotropic hormone (ACTH), supporting an autocrine-paracrine role (Petraglia, 1999). Large amounts of trophoblast CRH enter maternal blood.

Other proposed biological roles include induction of smooth-muscle relaxation in vascular and myometrial tissue and immunosuppression. The physiological reverse, however, induction of myometrial contractions, has been proposed for the rising CRH levels seen near term. One hypothesis suggests that CRH may be involved with parturition initiation (Wadhwa, 1998). Some evidence suggests that urocortin 2 expression is induced at term and induces expression of proinflammatory markers and prostaglandin F receptor expression in the placenta and myometrium (Voltolini, 2015). Prostaglandin formation in the placenta, amnion, chorion laeve, and decidua is increased with CRH treatment (Jones, 1989b). These observations further support a potential action in parturition timing.

Glucocorticoids act in the hypothalamus to inhibit CRH release, but in the trophoblast, glucocorticoids stimulate CRH gene expression (Jones, 1989a; Robinson, 1988). Thus, there may be a novel positive feedback loop in the placenta by which placental CRH stimulates placental ACTH to stimulate fetal and maternal adrenal glucocorticoid production with subsequent stimulation of placental CRH expression (Nicholson, 2001; Riley, 1991).

G*rowth hormone-releasing hormone* has an unknown role (Berry, 1992). Ghrelin is another regulator of hGH secretion that is produced by placental tissue (Horvath, 2001). Trophoblast ghrelin expression peaks at midpregnancy and is a paracrine regulator of differentiation or is a potential regulator of human growth hormone variant production, described next (Fuglsang, 2005; Gualillo, 2001).

Pituitary-Like Hormones

ACTH, lipotropin, and β-endorphin, which are all proteolytic products of pro-opiomelanocortin, are recovered from placental extracts (Genazzani, 1975; Odagiri, 1979). The physiological action of placental ACTH is unclear. As discussed, placental CRH stimulates synthesis and release of chorionic ACTH.

A *human growth hormone variant (hGH-V)* that is not expressed in the pituitary is expressed in the placenta. The gene encoding hGH-V is located in the hGH–hPL gene cluster on chromosome 17. Sometimes referred to as placental growth hormone, hGH-V is a 191-amino-acid protein that differs in 15 amino acid positions from the sequence for hGH. Although hGH-V retains growth-promoting and antilipogenic functions similar to those of hGH, it has reduced diabetogenic and lactogenic functions relative to hGH (Vickers, 2009). Placental hGH-V presumably is synthesized in the syncytiotrophoblast. It is believed that hGH-V is present in maternal plasma by 21 to 26 weeks' gestation, rises in concentration until approximately 36 weeks, and remains relatively constant thereafter. There is a correlation between the levels of hGH-V in maternal plasma and those of insulin-like growth factor-1. Also, hGH-V secretion by trophoblast in vitro is inhibited by glucose in a dose-dependent manner (Patel, 1995). Overexpression of hGH-V in mice causes severe insulin resistance, making it a likely candidate to mediate insulin resistance of pregnancy (Liao, 2016).

Relaxin

Expression of relaxin has been demonstrated in human corpus luteum, decidua, and placenta (Bogic, 1995). This peptide is synthesized as a single, 105-amino-acid preprorelaxin molecule that is cleaved to A and B molecules. Relaxin is structurally similar to insulin and insulin-like growth factor. Two of the three relaxin genes—*H2* and *H3*—are transcribed in the corpus luteum (Bathgate, 2002; Hudson, 1983, 1984). Decidua, placenta, and membranes express *H1* and *H2* (Hansell, 1991).

The rise in maternal circulating relaxin levels seen in early pregnancy is attributed to corpus luteum secretion, and levels parallel those of hCG. Relaxin, along with rising progesterone levels, may act on myometrium to promote relaxation and the quiescence of early pregnancy (Chap. 21, Decidua). In addition, the production of relaxin and relaxin-like factors within the placenta and fetal membranes may play an autocrine-paracrine role in postpartum regulation of extracellular matrix remodeling (Qin, 1997a,b). One important relaxin function is enhancement of the glomerular filtration rate (Chap. 4, Renal Function Tests).

Parathyroid Hormone-Related Protein

In pregnancy, circulating parathyroid hormone-related protein (PTH-rP) levels are significantly elevated within maternal but not fetal circulation (Bertelloni, 1994; Saxe, 1997). Many functions of this hormone have been proposed. PTH-rP synthesis is found in several normal adult tissues, especially in reproductive organs that include myometrium, endometrium, corpus luteum, and lactating mammary tissue. PTH-rP is not produced in the parathyroid glands of normal adults. Placenta-derived PTH-rP may have an important function to regulate genes involved in transfer of calcium and other solutes. It also contributes to fetal mineral homeostasis in bone, amnionic fluid, and the fetal circulation (Simmonds, 2010).

Leptin

This hormone is normally secreted by adipocytes. It functions as an antiobesity hormone that decreases food intake through its hypothalamic receptor. It also regulates bone growth and immune function (Cock, 2003; La Cava, 2004). In the placenta, leptin is synthesized by both cytotrophoblasts and syncytiotrophoblast (Henson, 2002). Relative contributions of leptin from maternal adipose tissue versus placenta are currently not well defined, although recent evidence highlights a key regulatory role of placental leptin in placental amino acid transport and fetal growth (Rosario, 2016a). Maternal serum levels are significantly higher than those in nonpregnant women. Fetal leptin levels correlate positively with birthweight and likely function in fetal development and growth. Studies suggest that reductions in leptin availability contribute to adverse fetal metabolic programing in intrauterine growth-restricted offspring (Nusken, 2016).

Neuropeptide Y

This 36-amino-acid peptide is widely distributed in brain. It also is found in sympathetic neurons innervating the cardiovascular, respiratory, gastrointestinal, and genitourinary systems. Neuropeptide Y has been isolated from the placenta and localized in cytotrophoblasts (Petraglia, 1989). Trophoblasts possess neuropeptide Y receptors, and treatment of these with neuropeptide Y causes CRH release (Robidoux, 2000).

Inhibin and Activin

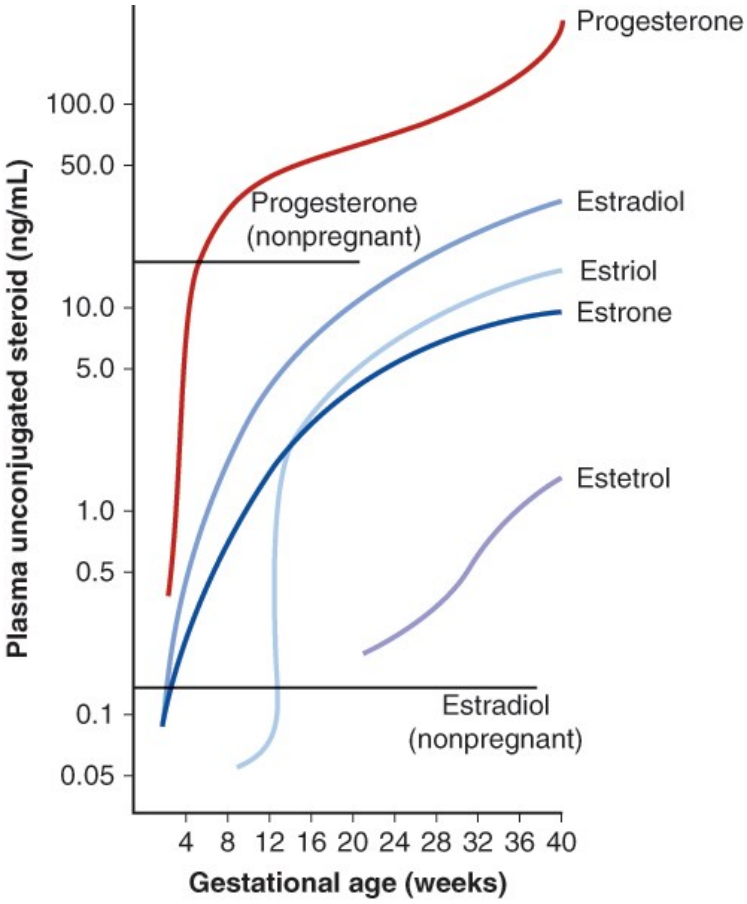
These glycoprotein hormones are expressed in male and female reproductive tissues and belong to the transforming growth factor-β family (Jones, 2006). Inhibin is a heterodimer made up of one α-subunit and one of two distinct β-subunits, either βA or βB. This yields either inhibin A or inhibin B, respectively. Activin is formed by the combination of the two β-subunits. Activin, inhibin, and their respective receptors are expressed in the placenta. Both activin and inhibin A have proposed functions during cytotrophoblast fusion into the syncytiotrophoblast (Debiève, 2000; Jones, 2006). Activin also stimulates production of placental hormones such as hCG, hPL, progesterone, and estrogen (Luo, 2002; Morrish, 1991; Petraglia, 1989; Song, 1996). Inhibin A opposes activin action in the placenta to inhibit production of hCG and steroidogenesis (Petraglia, 1989). Abnormal levels of inhibin or activin correlate with placental pathologies. For example, elevation in inhibin A levels in the second trimester is indicative of fetal Down syndrome. Further, low inhibin levels early in pregnancy may indicate pregnancy failure (Prakash, 2005; Wallace, 1996). Elevations in circulating inhibin and activin levels are reported in women with preeclampsia (Bersinger, 2003).

Placental Progesterone Production

After 6 to 7 weeks' gestation, little progesterone is produced in the ovary (Diczfalusy, 1961). Surgical removal of the corpus luteum or even bilateral oophorectomy during the 7th to 10th week does not decrease excretion rates of urinary pregnanediol, the principal urinary metabolite of progesterone. Before this time, however, corpus luteum removal will result in spontaneous abortion unless an exogenous progestin is given (Chap. 63, Diagnosis). After approximately 8 weeks, the placenta assumes progesterone secretion, resulting in a gradual increase in maternal serum levels throughout pregnancy (Fig. 5-19). By term, these levels are 10 to 5000 times those found in nonpregnant women, depending on the stage of the ovarian cycle.

FIGURE 5-19

Plasma levels of progesterone, estradiol, estrone, estetrol, and estriol in women during the course of gestation. (Modified and redrawn with permission from Mesiano S: The endocrinology of human pregnancy and fetoplacental neuroendocrine development. In Strauss JF, Barbieri RL (eds) Yen and Jaffe's Reproductive Endocrinology: Physiology, Pathophysiology, and Clinical Management, 6th ed. Philadephia, Saunders, 2009.)



Source: F. Gary Cunningham, Kenneth J. Leveno, Steven L. Bloom, Catherine Y. Spong, Jodi S. Dashe, Barbara L. Hoffman, Brian M. Casey, Jeanne S. Sheffield: *Williams Obstetrics*, 25th Edition

The daily production rate of progesterone in late, normal, singleton pregnancies approximates 250 mg. In multifetal pregnancies, the daily production rate may exceed 600 mg. Progesterone is synthesized from cholesterol in a two-step enzymatic reaction. First, cholesterol is converted to pregnenolone within the mitochondria, in a reaction catalyzed by cytochrome P450 cholesterol side-chain cleavage enzyme. Pregnenolone leaves the mitochondria and is converted to progesterone in the endoplasmic reticulum by 3β-hydroxysteroid dehydrogenase. Progesterone is released immediately through a process of diffusion.

Although the placenta produces a prodigious amount of progesterone, the syncytiotrophoblast has a limited capacity for cholesterol biosynthesis. Radiolabeled acetate is incorporated into cholesterol by placental tissue at a slow rate. The rate-limiting enzyme in cholesterol biosynthesis is 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase. Because of this, the placenta must rely on an exogenous source, that is, maternal cholesterol, for progesterone formation. The trophoblast preferentially uses LDL cholesterol for progesterone biosynthesis (Simpson, 1979, 1980).

This mechanism differs from placental production of estrogens, which relies principally on fetal adrenal precursors.

Although there is a relationship between fetal well-being and placental estrogen production, this is not the case for placental progesterone. Thus, placental endocrine function, including the formation of protein hormones such as hCG and progesterone biosynthesis, may persist for weeks after fetal demise.

The metabolic clearance rate of progesterone in pregnant women is similar to that found in men and nonpregnant women. During pregnancy, the plasma concentration of 5α-dihydroprogesterone disproportionately rises due to synthesis in syncytiotrophoblast from both placenta-produced progesterone and fetus-derived precursor (Dombroski, 1997). Thus, the concentration ratio of this progesterone metabolite to progesterone is elevated in pregnancy. The mechanisms for this are not defined completely. Progesterone also is converted to the potent mineralocorticoid deoxycorticosterone in pregnant women and in the fetus. The concentration of deoxycorticosterone is strikingly higher in both maternal and fetal compartments (see Table 5-1). The extraadrenal formation of deoxycorticosterone from circulating progesterone accounts for most of its production in pregnancy (Casey, 1982a,b).

Placental Estrogen Production

During the first 2 to 4 weeks of pregnancy, rising hCG levels maintain production of estradiol in the maternal corpus luteum. Production of both progesterone and estrogens in the maternal ovaries drops significantly by the 7th week of pregnancy. At this time, there is a luteal–placental transition. By the 7th week, more than half of estrogen entering maternal circulation is produced in the placenta (MacDonald, 1965a; Siiteri, 1963, 1966). Subsequently, the placenta produces a continually increasing magnitude of estrogen. Near term, normal human pregnancy is a hyperestrogenic state, and syncytiotrophoblast is producing estrogen in amounts equivalent to that produced in 1 day by the ovaries of no fewer than 1000 ovulatory women. This hyperestrogenic state terminates abruptly after delivery of the placenta.

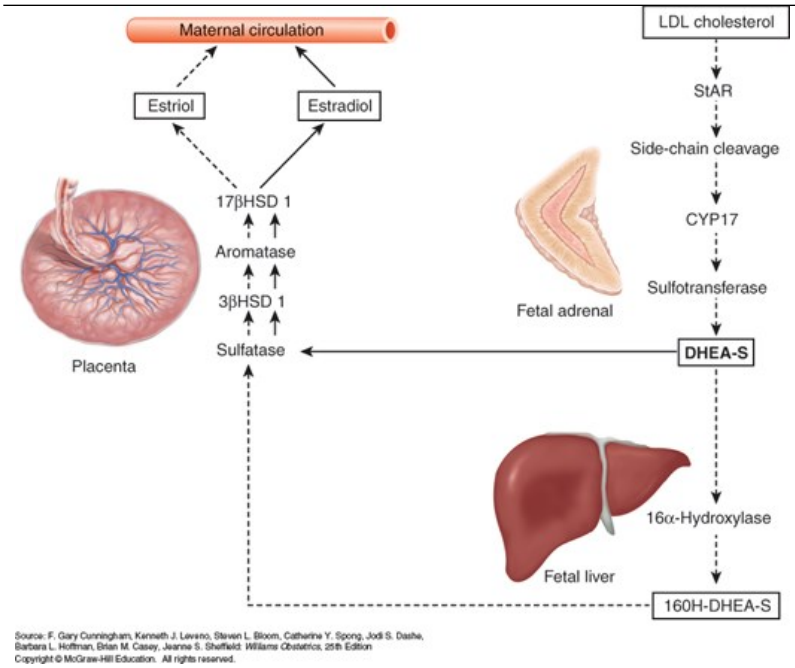
Biosynthesis

In human trophoblast, neither cholesterol nor, in turn, progesterone can serve as precursor for estrogen biosynthesis. This is because *steroid 17α-hydroxylase/17,20-lyase (CYP17A1)* is not expressed in the human placenta. This essential enzyme converts 17-OH progesterone (a C_{21} steroid) to androstenedione, which is a C_{19} steroid and an estrogen precursor. Consequently, the conversion of C_{21} steroids to C_{19} steroids is not possible.

However, dehydroepiandrosterone (DHEA) and its sulfate (DHEA-S) are also C_{19} steroids and are produced by maternal and fetal adrenal glands. These two steroids can serve as estrogen precursors (Fig. 5-20). Ryan (1959a) found that the placenta had an exceptionally high capacity to convert appropriate C_{19} steroids to estrone and estradiol. The conversion of DHEA-S to estradiol requires placental expression of four key enzymes that are located principally in syncytiotrophoblast (Bonenfant, 2000; Salido, 1990). First, the placenta expresses high levels of steroid sulfatase (STS), which converts the conjugated DHEA-S to DHEA. DHEA is then acted upon by 3β-hydroxysteroid dehydrogenase type 1 (3βHSD) to produce androstenedione. Cytochrome P450 aromatase (CYP19) then converts androstenedione to estrone, which is then converted to estradiol by 17β-hydroxysteroid dehydrogenase type 1 (17βHSD1).

FIGURE 5-20

Schematic presentation of estrogen biosynthesis in the human placenta. Dehydroepiandrosterone sulfate (DHEAS), secreted in prodigious amounts by the fetal adrenal glands, is converted to 16α-hydroxydehydroepiandrosterone sulfate (16αOHDHEAS) in the fetal liver. These steroids, DHEAS and 16αOHDHEAS, are converted in the placenta to estrogens, that is, 17β-estradiol (E_2) and estriol (E_3). Near term, half of E_2 is derived from fetal adrenal DHEAS and half from maternal DHEAS. On the other hand, 90 percent of E_3 in the placenta arises from fetal 16αOHDHEAS and only 10 percent from all other sources.



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DHEA-S is the major precursor of estrogens in pregnancy (Baulieu, 1963; Siiteri, 1963). However, maternal adrenal glands do not produce sufficient amounts of DHEA-S to account for more than a fraction of total placental estrogen biosynthesis. The fetal adrenal glands are quantitatively the most important source of placental estrogen precursors in human pregnancy. Thus, estrogen production during pregnancy reflects the unique interactions among fetal adrenal glands, fetal liver, placenta, and maternal adrenal glands.

Directional Secretion

More than 90 percent of estradiol and estriol formed in syncytiotrophoblast enters maternal plasma (Gurpide, 1966). And, 85 percent or more of placental progesterone enters maternal plasma, and little maternal progesterone crosses the placenta to the fetus (Gurpide, 1972).

This directional movement of newly formed steroid into the maternal circulation stems from basic characteristics of hemochorioendothelial placentation. In this system, steroids secreted from syncytiotrophoblast can enter maternal blood directly. Steroids that leave the syncytium do not enter fetal blood directly. They must first traverse the cytotrophoblast layer and then enter the stroma of the villous core and then fetal capillaries. From either of these spaces, steroids can reenter the syncytium. The net result of this hemochorial arrangement is that entry of steroids into the maternal circulation is substantially greater than that into fetal blood.

FETAL ADRENAL GLAND–PLACENTAL INTERACTIONS

Morphologically, functionally, and physiologically, the fetal adrenal glands are remarkable. At term, the fetal adrenal glands weigh the same as those of the adult. More than 85 percent of the fetal gland is composed of a unique fetal zone, which has a great capacity for steroid biosynthesis. Daily steroid production of fetal adrenal glands near term is 100 to 200 mg/d. This compares with resting adult steroid secretion of 30 to 40 mg/d.

The fetal zone is lost in the first year of life and is not present in the adult. In addition to ACTH, fetal adrenal gland growth is influenced by factors secreted by the placenta. This is exemplified by the continued growth of the fetal glands throughout gestation and by rapid involution immediately after birth and placental delivery.

Placental Estriol Synthesis

Estradiol is the primary placental estrogen product at term. In addition, significant levels of *estriol* and *estetrol* are found in the maternal circulation, and levels also rise, particularly late in gestation (see Fig. 5-19). These hydroxylated forms of estrogen derive from the placenta using substrates formed by the combined efforts of the fetal adrenal gland and fetal liver. For this, high levels of fetal hepatic 16α-hydroxylase act on adrenal-derived steroids. Ryan (1959b) and MacDonald and Siiteri (1965b) found that 16α-hydroxylated C_{19} steroids, particularly 16α-hydroxydehydroepiandrosterone

(16-OHDHEA), were converted to estriol by placental tissue. Thus, the disproportionate increase in estriol formation during pregnancy is accounted for by placental synthesis of estriol principally from plasma-borne 16-OHDHEA-sulfate. Near term, the fetus is the source of 90 percent of placental estriol and estetrol precursors in normal human pregnancy.

Maternal estriol and estetrol are produced almost solely by fetal steroid precursors. Thus, in the past, levels of these steroids were used as an indicator of fetal well-being. However, the low sensitivity and specificity of such tests have caused them to be discarded.

Fetal Adrenal Steroid Precursor

The precursor for fetal adrenal steroidogenesis is cholesterol. The steroid biosynthesis rate in the fetal gland is so great that its steroidogenesis alone is equivalent to a fourth of the total daily LDL cholesterol turnover in adults. Fetal adrenal glands synthesize cholesterol from acetate. All enzymes involved in cholesterol biosynthesis are elevated compared with those of the adult adrenal gland (Rainey, 2001). Thus, the de novo cholesterol synthesis rate by fetal adrenal tissue is extremely high. Even so, it is insufficient to account for the steroids produced by fetal adrenal glands. Therefore, cholesterol must be assimilated from the fetal circulation and mainly from LDL (Carr, 1980, 1981b, 1982; Simpson, 1979).

Most fetal plasma cholesterol arises by de novo synthesis in the fetal liver (Carr, 1984). The low LDL cholesterol level in fetal plasma is not the consequence of impaired fetal LDL synthesis, but instead results from the rapid use of LDL by the fetal adrenal glands for steroidogenesis (Parker, 1980, 1983).

Fetal Conditions Affecting Estrogen Production

Several fetal disorders alter the availability of substrate for placental steroid synthesis and thus highlight the interdependence of fetal development and placental function.

Fetal demise is followed by a striking reduction in urinary estrogen levels. Similarly, after ligation of the umbilical cord with the fetus and placenta left in situ, placental estrogen production declines markedly (Cassmer, 1959). However, as previously discussed, placental progesterone production is maintained. In sum, an important source of precursors of placental estrogen—but not progesterone—biosynthesis is eliminated with fetal death.

Anencephalic fetuses have markedly atrophic adrenal glands. This stems from absent hypothalamic-pituitary function, which precludes adrenal stimulation by ACTH. With absence of the adrenal cortex fetal zone, the placental formation of estrogen—especially estriol—is severely limited because of diminished availability of C_{19} steroid precursors. Indeed, urinary estrogen levels in women pregnant with an anencephalic fetus are only about 10 percent of those found in normal pregnancy (Frandsen, 1961). With an anencephalic fetus, almost all estrogens produced arise from placental use of maternal plasma DHEA-S.

Fetal adrenal cortical hypoplasia occurs in perhaps 1 in 12,500 births (McCabe, 2001). Estrogen production in these pregnancies is limited, which suggests the absence of C_{19} precursors.

Fetal–placental sulfatase deficiency is associated with very low estrogen levels in otherwise normal pregnancies (France, 1969). Namely, sulfatase deficiency precludes the hydrolysis of C_{19} steroid sulfates, the first enzymatic step in the placental use of these circulating prehormones for estrogen biosynthesis. This deficiency is an X-linked disorder, and thus all affected fetuses are male. Its estimated frequency is 1 in 2000 to 5000 births and is associated with delayed labor onset. It also is associated with the development of ichthyosis in affected males later in life (Bradshaw, 1986).

Fetal–placental aromatase deficiency is a rare autosomal recessive disorder in which individuals cannot synthesize endogenous estrogens (Grumbach, 2011; Simpson, 2000). To recall, fetal adrenal DHEA-S is converted in the placenta to androstenedione, but in cases of placental aromatase deficiency, androstenedione cannot be converted to estradiol. Rather, androgen metabolites of DHEA produced in the placenta, including androstenedione and some testosterone, are secreted into the maternal or fetal circulation, or both. This can cause virilization of the mother and the female fetus (Belgorosky, 2009; Harada, 1992; Shozu, 1991).

Trisomy 21—Down syndrome screening searches for abnormal levels of hCG, alpha-fetoprotein, and other analytes (Chap. 14, Traditional Aneuploidy Screening Tests). It was discovered that serum unconjugated estriol levels were low in women with Down syndrome fetuses (Benn, 2002). The likely reason for this is inadequate formation of C_{19} steroids in the adrenal glands of these trisomic fetuses.

Fetal erythroblastosis in some cases of severe fetal D-antigen alloimmunization can lead to elevated maternal plasma estrogen levels. A suspected cause is the greater placental mass from hypertrophy, which can be seen with such fetal hemolytic anemia (Chap. 15, Fetal Anemia).

Maternal Conditions Affecting Estrogen Production

Glucocorticoid treatment can cause a striking reduction in placental estrogen formation. Glucocorticoids inhibit ACTH secretion from the maternal and fetal pituitary glands. This diminishes maternal and fetal adrenal secretion of the placental estrogen precursor DHEA-S.

With *Addison disease,* pregnant women show lower estrogen levels, principally estrone and estradiol levels (Baulieu, 1956). The fetal adrenal contribution to estriol synthesis, particularly in later pregnancy, is quantitatively much more important.

Maternal *androgen-producing tumors* can present the placenta with elevated androgen levels. Fortunately, placenta is extraordinary efficient in the aromatization of C_{19} steroids. For example, Edman and associates (1981) found that virtually all androstenedione entering the intervillous space is taken up by syncytiotrophoblast and converted to estradiol. None of this C_{19} steroid enters the fetus. Second, a female fetus is rarely virilized if there is a maternal androgen-secreting tumor. The placenta efficiently converts aromatizable C_{19} steroids, including testosterone, to estrogens, thus precluding transplacental passage. Indeed, virilized female fetuses of women with an androgen-producing tumor may be cases in which a nonaromatizable C_{19} steroid androgen is produced by the tumor—for example, 5α-dihydrotestosterone. Another explanation is that testosterone is produced very early in pregnancy in amounts that exceed the placental aromatase capacity at that time.

Complete hydatidiform mole and *gestational trophoblastic neoplasias* lack a fetus and also a fetal adrenal source of C_{19} steroid precursors for trophoblast estrogen biosynthesis. Consequently, placental estrogen formation is limited to the use of C_{19} steroids from the maternal plasma, and therefore estradiol is principally produced (MacDonald, 1964, 1966).

REFERENCES

Abel MH: Prostanoids and menstruation. In Baird DT, Michie EA (eds): Mechanisms of Menstrual Bleeding. New York, Raven, 2002

Albrecht ED, Pepe GJ: Steroid hormone regulation of angiogenesis in the primate endometrium. Front Biosci 8:D416, 2003

Ancelin M, Buteau-Lozano H, Meduri G, et al: A dynamic shift of VEGF isoforms with a transient and selective progesterone-induced expression of VEGF189 regulates angiogenesis and vascular permeability in human uterus. Proc Natl Acad Sci USA 99:6023, 2002

Aplin JD: MUC-1 glycosylation in endometrium: possible roles of the apical glycocalyx at implantation. Hum Reprod 2:17, 2003

Apps R, Sharkey A, Gardner L, et al: Ex vivo functional responses to HLA-G differ between blood and decidual NK cells. Mol Hum Reprod 17(9):577, 2011

Arici A, Head JR, MacDonald PC, et al: Regulation of interleukin-8 gene expression in human endometrial cells in culture. Mol Cell Endocrinol 94:195, 1993

Arici A, MacDonald PC, Casey ML: Regulation of monocyte chemotactic protein-1 gene expression in human endometrial cells in cultures. Mol Cell Endocrinol 107:189, 1995

Arnholdt H, Meisel F, Fandrey K, et al: Proliferation of villous trophoblast of the human placenta in normal and abnormal pregnancies. Virchows Arch B Cell Pathol Incl Mol Pathol 60:365, 1991

Baker T: A quantitative and cytological study of germ cells in human ovaries. Proc R Soc Lond B Biol Sci 158:417, 1963

Bathgate RA, Samuel CS, Burazin TC, et al: Human relaxin gene 3 (H3) and the equivalent mouse relaxin (M3) gene. Novel members of the relaxin peptide family. J Biol Chem 277:1148, 2002

Baulieu EE, Bricaire H, Jayle MF: Lack of secretion of 17-hydroxycorticosteroids in a pregnant woman with Addison's disease. J Clin Endocrinol 16:690, 1956

Baulieu EE, Dray F: Conversion of 3H-dehydroepiandrosterone (3Beta-hydroxy-delta5-androsten-17-one) sulfate to 3H-estrogens in normal pregnant women. J Clin Endocrinol 23:1298, 1963

Beck T, Schweikhart G, Stolz E: Immunohistochemical location of HPL, SP1 and beta-HCG in normal placentas of varying gestational age. Arch Gynecol 239:63, 1986

Belgorosky A, Guercio G, Pepe C, et al: Genetic and clinical spectrum of aromatase deficiency in infancy, childhood and adolescence. Horm Res 72(6):321, 2009

Benirschke K, Burton GJ, Baergen RN: Pathology of the Human Placenta, 6th ed. Heidelberg, Springer, 2012

Benn PA: Advances in prenatal screening for Down syndrome: I. General principles and second trimester testing. Clin Chim Acta 323:1, 2002

Berry SA, Srivastava CH, Rubin LR, et al: Growth hormone-releasing hormone-like messenger ribonucleic acid and immunoreactive peptide are present in human testis and placenta. J Clin Endocrinol Metab 75:281, 1992

Bersinger NA, Smarason AK, Muttukrishna S, et al: Women with preeclampsia have increased serum levels of pregnancy-associated plasma protein A (PAPP-A), inhibin A, activin A and soluble E-selectin. Hypertens Pregnancy 22(1):45, 2003

Bertelloni S, Baroncelli GI, Pelletti A, et al: Parathyroid hormone-related protein in healthy pregnant women. Calcif Tissue Int 54:195, 1994

Bhaumick B, Dawson EP, Bala RM: The effects of insulin-like growth factor-I and insulin on placental lactogen production by human term placental explants. Biochem Biophys Res Commun 144:674, 1987

Bleker O, Kloostermans G, Mieras D, et al: Intervillous space during uterine contractions in human subjects: an ultrasonic study. Am J Obstet Gynecol 123:697, 1975

Bogic LV, Mandel M, Bryant-Greenwood GD: Relaxin gene expression in human reproductive tissues by in situ hybridization. J Clin Endocrinol Metab 80:130, 1995

Bonagura TW, Babischkin JS, Aberdeen GC, et al: Prematurely elevating estradiol in early baboon pregnancy suppresses uterine artery remodeling and expression of extravillous placental vascular endothelial growth factor and a1b1 integrins. Endocrinology 153(6):2897, 2012

Bonenfant M, Provost PR, Drolet R, et al: Localization of type 1 17beta-hydroxysteroid dehydrogenase mRNA and protein in syncytiotrophoblasts and invasive cytotrophoblasts in the human term villi. J Endocrinol 165:217, 2000

Borell U, Fernstrom I, Westman A: An arteriographic study of the placental circulation. Geburtshilfe Frauenheilkd 18:1, 1958

Bourne GL: The Human Amnion and Chorion. Chicago, Year Book, 1962

Boyd JD, Hamilton WJ: The Human Placenta. Cambridge, England, Heffer, 1970

Bradshaw KD, Carr BR: Placental sulfatase deficiency: maternal and fetal expression of steroid sulfatase deficiency and X-linked ichthyosis. Obstet Gynecol Surv 41:401, 1986

Brosens I, Dixon H: The anatomy of the maternal side of the placenta. Eur J Endocrinol 73:357, 1963

Bryant-Greenwood GD: The extracellular matrix of the human fetal membranes: structure and function. Placenta 19:1, 1998

Carr BR, Ohashi M, Simpson ER: Low density lipoprotein binding and de novo synthesis of cholesterol in the neocortex and fetal zones of the human fetal adrenal gland. Endocrinology 110:1994, 1982

Carr BR, Porter JC, MacDonald PC, et al: Metabolism of low density lipoprotein by human fetal adrenal tissue. Endocrinology 107:1034, 1980

Carr BR, Sadler RK, Rochelle DB, et al: Plasma lipoprotein regulation of progesterone biosynthesis by human corpus luteum tissue in organ culture. J Clin Endocrinol Metab 52:875, 1981a

Carr BR, Simpson ER: Cholesterol synthesis by human fetal hepatocytes: effect of lipoproteins. Am J Obstet Gynecol 150:551, 1984

Carr BR, Simpson ER: Lipoprotein utilization and cholesterol synthesis by the human fetal adrenal gland. Endocr Rev 2:306, 1981b

Carson DD: The glycobiology of implantation. Front Biosci 7:d1535, 2002

Carvajal JA, Delpiano AM, Cuello MA, et al: Mechanical stretch increases brain natriuretic peptide production and secretion in the human fetal membranes. Reprod Sci 20(5):597, 2013

Casey ML, Delgadillo M, Cox KA, et al: Inactivation of prostaglandins in human decidua vera (parietalis) tissue: substrate specificity of prostaglandin dehydrogenase. Am J Obstet Gynecol 160:3, 1989

Casey ML, Hemsell DL, MacDonald PC, et al: NAD^+-dependent 15-hydroxyprostaglandin dehydrogenase activity in human endometrium. Prostaglandins 19:115, 1980

Casey ML, MacDonald PC: Extraadrenal formation of a mineralocorticosteroid: deoxycorticosterone and deoxycorticosterone sulfate biosynthesis and metabolism. Endocr Rev 3:396, 1982a

Casey ML, MacDonald PC: Metabolism of deoxycorticosterone and deoxycorticosterone sulfate in men and women. J Clin Invest 70:312, 1982b

Casey ML, MacDonald PC: The endothelin-parathyroid hormone-related protein vasoactive peptide system in human endometrium: modulation by transforming growth factor-beta. Hum Reprod 11(Suppl 2):62, 1996

Cassmer O: Hormone production of the isolated human placenta. Studies on the role of the foetus in the endocrine functions of the placenta. Acta Endocrinol Suppl 32(Suppl 45):1, 1959

Cervar M, Blaschitz A, Dohr G, et al: Paracrine regulation of distinct trophoblast functions in vitro by placental macrophages. Cell Tissue Res 295:297, 1999

Chennazhi, Nayak NR: Regulation of angiogenesis in the primate endometrium: vascular endothelial growth factor. Semin Reprod Med 27(1):80, 2009

Chigusa Y, Kishore AH, Mogami H, et al: Nrf2 activation inhibits effects of thrombin in human amnion cells and thrombin-induced preterm birth in mice. J Clin Endocrinol Metab 101(6):2612, 2016

Cock TA, Auwerx J: Leptin: cutting the fat off the bone. Lancet 362:1572, 2003

Cole LA: Immunoassay of human chorionic gonadotropin, its free subunits, and metabolites. Clin Chem 43:2233, 1997

Conneely OM, Mulac-Jericevic B, DeMayo F, et al: Reproductive functions of progesterone receptors. Recent Prog Horm Res 57:339, 2002

Corbacho AM, Martinez DL, Clapp C: Roles of prolactin and related members of the prolactin/growth hormone/placental lactogen family in angiogenesis. J Endocrinol 173:219, 2002

Coya R, Martul P, Algorta J, et al: Progesterone and human placental lactogen inhibit leptin secretion on cultured trophoblast cells from human placentas at term. Gynecol Endocrinol 21:27, 2005

Crawford J: A study of human placental growth with observations on the placenta in erythroblastosis foetalis. BJOG 66:855, 1959

Critchley HO, Kelly RW, Baird DT, et al: Regulation of human endometrial function: mechanisms relevant to uterine bleeding. Reprod Biol Endocrinol 4 Suppl 1:S5, 2006

Curry TE Jr, Smith MF: Impact of extracellular matrix remodeling on ovulation and the folliculo-luteal transition. Semin Reprod Med 24(4):228, 2006

Dautzenberg FM, Hauger RL: The CRF peptide family and their receptors: yet more partners discovered. Trends Pharmacol Sci 23:71, 2002

Debiève F, Pampfer S, Thomas K: Inhibin and activin production and subunit expression in human placental cells cultured *in vitro*. Mol Human Reprod 6(8):743, 2000

Diczfalusy E, Troen P: Endocrine functions of the human placenta. Vitam Horm 19:229, 1961

Dombroski RA, Casey ML, MacDonald PC: 5-Alpha-dihydroprogesterone formation in human placenta from 5alpha-pregnan-3beta/alpha-ol-20-ones and 5-pregnan-3beta-yl-20-one sulfate. J Steroid Biochem Mol Biol 63:155, 1997

Duffy DM, Hutchison JS, Stewart DR, et al: Stimulation of primate luteal function by recombinant human chorionic gonadotropin and modulation of steroid, but not relaxin, production by an inhibitor of 3 beta-hydroxysteroid dehydrogenase during simulated early pregnancy. J Clin Endocrinol Metab 81:2307, 1996

Duncan WC, McNeilly AS, Fraser HM, et al: Luteinizing hormone receptor in the human corpus luteum: lack of down-regulation during maternal recognition of pregnancy. Hum Reprod 11:2291, 1996

Dunn CL, Critchley HO, Kelly RW: IL-15 regulation in human endometrial stromal cells. J Clin Endocrinol Metab 87:1898, 2002

Economos K, MacDonald PC, Casey ML: Endothelin-1 gene expression and protein biosynthesis in human endometrium: potential modulator of endometrial blood flow. J Clin Endocrinol Metab 74:14, 1992

Edman CD, Toofanian A, MacDonald PC, et al: Placental clearance rate of maternal plasma androstenedione through placental estradiol formation: an indirect method of assessing uteroplacental blood flow. Am J Obstet Gynecol 141:1029, 1981

Elliott CL, Allport VC, Loudon JA, et al: Nuclear factor-kappa B is essential for up-regulation of interleukin-8 expression in human amnion and cervical epithelial cells. Mol Hum Reprod 7:787, 2001

Evans J, Salamonsen LA: Inflammation, leukocytes and menstruation. Rev Endocr Metab Disord 13(4):277, 2012

Faas MM, de Vos P: Uterine NK cells and macrophages in pregnancy. Placenta 56:44, 2017

Faddy MJ, Gosden RG, Gougeon A, et al: Accelerated disappearance of ovarian follicles in mid-life: implications for forecasting menopause. Hum Reprod 7:1342, 1992

Ferenczy A: Studies on the cytodynamics of human endometrial regeneration. I. Scanning electron microscopy. Am J Obstet Gynecol 124:64, 1976

Filicori M, Santoro N, Merriam GR, et al: Characterization of the physiological pattern of episodic gonadotropin secretion throughout the human menstrual cycle. J Clin Endocrinol Metab 62:1136, 1986

Fisk NM, MacLachlan N, Ellis C, et al: Absent end-diastolic flow in first trimester umbilical artery. Lancet 2:1256, 1988

Florio P, Franchini A, Reis FM, et al: Human placenta, chorion, amnion and decidua express different variants of corticotropin-releasing factor receptor messenger RNA. Placenta 21:32, 2000

Florio P, Mezzesimi A, Turchetti V, et al: High levels of human chromogranin A in umbilical cord plasma and amniotic fluid at parturition. J Soc Gynecol Investig 9:32, 2002

France JT, Liggins GC: Placental sulfatase deficiency. J Clin Endocrinol Metab 29:138, 1969

Frandsen VA, Stakemann G: The site of production of oestrogenic hormones in human pregnancy: hormone excretion in pregnancy with anencephalic foetus. Acta Endocrinol 38:383, 1961

Fraser HM, Wulff C: Angiogenesis in the primate ovary. Reprod Fertil Dev 13:557, 2001

Fu B, Li X, Sun R, et al: Natural killer cells promote immune tolerance by regulating inflammatory TH17 cells at the human maternal-fetal interface. Proc Natl Acad Sci USA 110(3):E231, 2013

Fuglsang J, Skjaerbaek C, Espelund U, et al: Ghrelin and its relationship to growth hormones during normal pregnancy. Clin Endocrinol (Oxf) 62(5):554, 2005

Fuzzi B, Rizzo R, Criscuoli L, et al: HLA-G expression in early embryos is a fundamental prerequisite for the obtainment of pregnancy. Eur J Immunol 32:311, 2002

Garcia-Velasco JA, Arici A: Chemokines and human reproduction. Fertil Steril 71:983, 1999

Gargett CE, Rogers PA: Human endometrial angiogenesis. Reproduction 121:181, 2001

Genazzani AR, Fraioli F, Hurlimann J, et al: Immunoreactive ACTH and cortisol plasma levels during pregnancy. Detection and partial purification of corticotrophin-like placental hormone: the human chorionic corticotrophin (HCC). Clin Endocrinol (Oxf) 4:1, 1975

Genbacev O, Ratkovic M, Kraincanic M, et al: Effect of prostaglandin PGE2alpha on the synthesis of placental proteins and human placental lactogen (HPL). Prostaglandins 13:723, 1977

Georgia S, Bhushan A: Pregnancy hormones boost beta cells via serotonin. Nat Med 16(7):756, 2010

Germain A, Attaroglu H, MacDonald PC, et al: Parathyroid hormone-related protein mRNA in avascular human amnion. J Clin Endocrinol Metab 75:1173, 1992

Gibson DA, Simitsidelis I, Cousins FL, et al: Intracrine androgens enhance decidualization and modulate expression of human endometrial receptivity genes. Sci Rep 286, 2016

Golander A, Hurley T, Barrett J, et al: Prolactin synthesis by human chorion-decidual tissue: a possible source of prolactin in the amniotic fluid. Science 202:311, 1978

Goldman-Wohl DS, Ariel I, Greenfield C, et al: HLA-G expression in extravillous trophoblasts is an intrinsic property of cell differentiation: a lesson learned from ectopic pregnancies. Mol Hum Reprod 6:535, 2000

Gougeon A: Regulation of ovarian follicular development in primates: facts and hypotheses. Endocr Rev 17:121, 1996

Greer LG, Casey BM, Halvorson LM, et al: Antithyroid antibodies and parity: further evidence for microchimerism in autoimmune thyroid disease. Am J Obstet Gynecol 205(5):471.e1, 2011

Groome NP, Illingworth PJ, O'Brien M, et al: Measurement of dimeric inhibin B throughout the human menstrual cycle. J Clin Endocrinol Metab 81:1401, 1996

Grumbach MM: Aromatase deficiency and its consequences. Adv Exp Med Biol 707:19, 2011

Grumbach MM, Kaplan SL: On placental origin and purification of chorionic growth hormone prolactin and its immunoassay in pregnancy. Trans N Y Acad Sci 27:167, 1964

Gualillo O, Caminos J, Blanco M, et al: Ghrelin, a novel placental-derived hormone. Endocrinology 142:788, 2001

Gubbay O, Critchley HO, Bowen JM, et al: Prolactin induces ERK phosphorylation in epithelial and CD56(+) natural killer cells of the human endometrium. J Clin Endocrinol Metab 87:2329, 2002

Gurpide E, Schwers J, Welch MT, et al: Fetal and maternal metabolism of estradiol during pregnancy. J Clin Endocrinol Metab 26:1355, 1966

Gurpide E, Tseng J, Escarcena L, et al: Fetomaternal production and transfer of progesterone and uridine in sheep. Am J Obstet Gynecol 113:21, 1972

Guzeloglu-Kayisli O, Kayisli UA, Taylor HS: The role of growth factors and cytokines during implantation: endocrine and paracrine interactions. Semin Reprod Med 27(1):62, 2009

Hafez ES, Ludwig H, Metzger H: Human endometrial fluid kinetics as observed by scanning electron microscopy. Am J Obstet Gynecol 122:929, 1975

Hanna J, Goldman-Wohl D, Hamani Y, et al: Decidual NK cells regulate key developmental processes at the human fetal-maternal interface. Nat Med 12:1065, 2006

Hansell DJ, Bryant-Greenwood GD, Greenwood FC: Expression of the human relaxin H1 gene in the decidua, trophoblast, and prostate. J Clin Endocrinol Metab 72:899, 1991

Harada N, Ogawa H, Shozu M, et al: Biochemical and molecular genetic analyses on placental aromatase (P-450AROM) deficiency. J Biol Chem 267:4781, 1992

Henson MC, Castracane VD: Leptin: roles and regulation in primate pregnancy. Semin Reprod Med 20:113, 2002

Hershman JM: Human chorionic gonadotropin and the thyroid: Hyperemesis gravidarum and trophoblastic tumors. Thyroid 9:653, 1999

Hertig AT: The placenta: some new knowledge about an old organ. Obstet Gynecol 20:859, 1962

Hillier SG: Gonadotropic control of ovarian follicular growth and development. Mol Cell Endocrinol 179:39, 2001

Horgan CE, Roumimper H, Tucker R, et al: Altered decorin and Smad expression in human fetal membranes in PPROM. Biol Reprod 91(5):105, 2014

Horvath TL, Diano S, Sotonyi P, et al: Minireview: ghrelin and the regulation of energy balance—a hypothalamic perspective. Endocrinology 142:4163, 2001

Hoshina M, Boothby M, Boime I: Cytological localization of chorionic gonadotropin alpha and placental lactogen mRNAs during development of the human placenta. J Cell Biol 93:190, 1982

Hreinsson JG, Scott JE, Rasmussen C, et al: Growth differentiation factor-9 promotes the growth, development, and survival of human ovarian follicles in organ culture. J Clin Endocrinol Metab 87:316, 2002

Hudson P, Haley J, John M, et al: Structure of a genomic clone encoding biologically active human relaxin. Nature 301:628, 1983

Hudson P, John M, Crawford R, et al: Relaxin gene expression in human ovaries and the predicted structure of a human preprorelaxin by analysis of cDNA clones. EMBO J 3:2333, 1984

Hunt JS, Orr HT: HLA and maternal-fetal recognition. FASEB J 6:2344, 1992

Iwashita M, Kudo Y, Shinozaki Y, et al: Gonadotropin-releasing hormone increases serum human chorionic gonadotropin in pregnant women. Endocr J 40:539, 1993

Jeffrey J: Collagen and collagenase: pregnancy and parturition. Semin Perinatol 15:118, 1991

Johnson EL, Chakraborty R: Placental Hofbauer cells limit HIV-1 replication and potentially offset mother to child transmission (MTCT) by induction of immunoregulatory cytokines. Retrovirology 9:101, 2012

Johnson PM, Christmas SE, Vince GS: Immunological aspects of implantation and implantation failure. Hum Reprod 14(suppl 2):26, 1999

Jones RL, Stoikos C, Findlay JK, et al: TGF-β superfamily expression and actions in the endometrium and placenta. Reproduction 132:217, 2006

Jones SA, Brooks AN, Challis JR: Steroids modulate corticotropin-releasing hormone production in human fetal membranes and placenta. J Clin Endocrinol Metab 68:825, 1989a

Jones SA, Challis JR: Local stimulation of prostaglandin production by corticotropin-releasing hormone in human fetal membranes and placenta. Biochem Biophys Res Commun 159:192, 1989b

Kaipia A, Hsueh AJ: Regulation of ovarian follicle atresia. Annu Rev Physiol 59:349, 1997

Kane N, Kelly R, Saunders PTK, et al: Proliferation of uterine natural killer cells is induced by hCG and mediated via the mannose receptor. Endocrinology 150(6):2882, 2009

Kaneko Y, Murphy CR, Day ML: Extracellular matrix proteins secreted from both the endometrium and the embryo are required for attachment: a study using a co-culture model of rat blastocysts and Ishikawa cells. J Morphol 2741(1):63, 2013

Katzenellenbogen BS, Sun J, Harrington WR, et al: Structure-function relationships in estrogen receptors and the characterization of novel selective estrogen receptor modulators with unique pharmacological profiles. Ann NY Acad Sci 949:6, 2001

Kaufmann P, Scheffen I: Placental development. In Polin R, Fox W (eds): Fetal and Neonatal Physiology. Philadelphia, Saunders, 1992

Kim H, Toyofuku Y, Lynn FC; et al: Serotonin regulates pancreatic beta cell mass during pregnancy. Nat Med 16(7):804, 2010

King BF, Menton DN: Scanning electron microscopy of human placental villi from early and late in gestation. Am J Obstet Gynecol 122:824, 1975

Kraiem Z, Sadeh O, Blithe DL, et al: Human chorionic gonadotropin stimulates thyroid hormone secretion, iodide uptake, organification, and adenosine 3′,5′-monophosphate formation in cultured human thyrocytes. J Clin Endocrinol Metab 79:595, 1994

Kurman RJ, Young RH, Norris HJ, et al: Immunocytochemical localization of placental lactogen and chorionic gonadotropin in the normal placenta and trophoblastic tumors, with emphasis on intermediate trophoblast and the placental site trophoblastic tumor. Int J Gynecol Pathol 3:101, 1984

Kurtzman JT, Wilson H, Rao CV: A proposed role for hCG in clinical obstetrics. Semin Reprod Med 19:63, 2001

La Cava A, Alviggi C, Matarese G: Unraveling the multiple roles of leptin in inflammation and autoimmunity. J Mol Med 82:4, 2004

LeBouteiller P, Solier C, Proll J, et al: Placental HLA-G protein expression in vivo: where and what for? Hum Reprod Update 5:223, 1999

Lessey BA, Castelbaum AJ: Integrins and implantation in the human. Rev Endocr Metab Disord 3:107, 2002

Lessey BA, Castelbaum AJ, Sawin SW, et al: Integrins as markers of uterine receptivity in women with primary unexplained infertility. Fertil Steril 63:535, 1995

Liao S, Vickers MH, Taylor RS, et al: Human placental growth hormone is increased in maternal serum at 20 weeks of gestation in pregnancies with large-for-gestation-age babies. Growth Factors 34(5–6):203, 2016

Licht P, Russu V, Wildt L: On the role of human chorionic gonadotropin (hCG) in the embryo-endometrial microenvironment: implications for differentiation and implantation. Semin Reprod Med 19:37, 2001

Lindhard A, Bentin-Ley U, Ravn V, et al: Biochemical evaluation of endometrial function at the time of implantation. Fertil Steril 78:221, 2002

Lobo SC, Srisuparp S, Peng X, et al: Uterine receptivity in the baboon: modulation by chorionic gonadotropin. Semin Reprod Med 19:69, 2001

Loke YM, King A: Human Implantation. Cell Biology and Immunology. Cambridge, Cambridge University Press, 1995

Loquet P, Broughton-Pipkin F, Symonds E, et al: Blood velocity waveforms and placental vascular formation. Lancet 2:1252, 1988

Luo S, Yu H, Wu D, et al: Transforming growth factor β1 inhibits steroidogenesis in human trophoblast cells. Molecular Human Reproduction 8(3):318, 2002

MacDonald PC: Placental steroidogenesis. In Wynn RM (ed): Fetal Homeostasis, Vol. I. New York, New York Academy of Sciences, 1965a

MacDonald PC, Siiteri PK: Origin of estrogen in women pregnant with an anencephalic fetus. J Clin Invest 44:465, 1965b

MacDonald PC, Siiteri PK: Study of estrogen production in women with hydatidiform mole. J Clin Endocrinol Metab 24:685, 1964

MacDonald PC, Siiteri PK: The in vivo mechanisms of origin of estrogen in subjects with trophoblastic tumors. Steroids 8:589, 1966

Macklon NS, Fauser BC: Follicle-stimulating hormone and advanced follicle development in the human. Arch Med Res 32:595, 2001

Manaster I, Mizrahi S, Goldman-Wohl D, et al: Endometrial NK cells are special immature cells that await pregnancy. J Immunol 181:1869, 2008

Marcellin L, Schmitz T, Messaoudene M, et al: Immune modifications in fetal membranes overlying the cervix precede parturition in humans. J Immunol 198(3):1345, 2017

Markee J: Menstruation in intraocular endometrial transplants in the rhesus monkey. Contrib Embryol 28:219, 1940

Maruo T, Ladines-Llave CA, Matsuo H, et al: A novel change in cytologic localization of human chorionic gonadotropin and human placental lactogen in first-trimester placenta in the course of gestation. Am J Obstet Gynecol 167:217, 1992

Maybin JA, Critchley HO: Menstrual physiology: implications for endometrial pathology and beyond. Hum Reprod Update 21(6):748, 2015

McCabe ER: Adrenal hypoplasias and aplasias. In Scriver CR, Beaudet AL, Sly WE, et al (eds): The Metabolic and Molecular Bases of Inherited Disease. New York, McGraw-Hill, 2001

McCombs H, Craig M: Decidual necrosis in normal pregnancy. Obstet Gynecol 24:436, 1964

McGregor WG, Kuhn RW, Jaffe RB: Biologically active chorionic gonadotropin: synthesis by the human fetus. Science 220:306, 1983

McGregor WG, Raymoure WJ, Kuhn RW, et al: Fetal tissue can synthesize a placental hormone. Evidence for chorionic gonadotropin beta-subunit

synthesis by human fetal kidney. J Clin Invest 68:306, 1981

Mesiano S: The endocrinology of human pregnancy and fetoplacental neuroendocrine development. In Strauss JF, Barbieri RL (eds): Yen and Jaffe's Reproductive Endocrinology: Physiology, Pathophysiology, and Clinical Management, 6th ed. Philadelphia, Saunders, 2009

Mi Y, Wang W, Zhang C, et al: Autophagic degradation of collagen 1A1 by cortisol in human amnion fibroblasts. Endocrinology 158(4):1005, 2017

Miller-Lindholm AK, LaBenz CJ, Ramey J, et al: Human chorionic gonadotropin-beta gene expression in first trimester placenta. Endocrinology 138:5459, 1997

Moffett-King A: Natural killer cells and pregnancy. Nat Rev Immunol 2:656, 2002

Mogami H, Keller PW, Shi H, et al: Effect of thrombin on human amnion mesenchymal cells, mouse fetal membranes, and preterm birth. J Biol Chem 289(19):13295, 2014

Mogami H, Kishore AH, Shi H, et al: Fetal fibronectin signaling induces matrix metalloproteases and cyclooxygenase-2 (COX-2) in amnion cells and preterm birth in mice. J Biol Chem 288(3):1953, 2013

Moore JJ, Dubyak GR, Moore RM, et al: Oxytocin activates the inositol-phospholipid-protein kinase-C system and stimulates prostaglandin production in human amnion cells. Endocrinology 123:1771, 1988

Moore KL, Persaud TV, Torchia MG (eds): The Developing Human. Clinically Oriented Embryology, 9th ed. Philadelphia, Saunders, 2013

Moore RM, Redline RW, Kumar D, et al: Differential expression of fibulin family proteins in the para-cervical weak zone and other areas of human fetal membranes. Placenta 30(4):335, 2009

Morrish DW, Bhardwaj D, Paras MT: Transforming growth factor beta 1 inhibits placental differentiation and human chorionic gonadotropin and human placental lactogen secretion. Endocrinology 129(1):22, 1991

Morrish DW, Marusyk H, Siy O: Demonstration of specific secretory granules for human chorionic gonadotropin in placenta. J Histochem Cytochem 35:93, 1987

Mote PA, Balleine RL, McGowan EM, et al: Colocalization of progesterone receptors A and B by dual immunofluorescent histochemistry in human endometrium during the menstrual cycle. J Clin Endocrinol Metab 84:2963, 1999

Mote PA, Balleine RL, McGowan EM, et al: Heterogeneity of progesterone receptors A and B expression in human endometrial glands and stroma. Hum Reprod 15(suppl 3):48, 2000

Navot D, Bergh P: Preparation of the human endometrium for implantation. Ann N Y Acad Sci 622:212, 1991

Nemeth E, Tashima LS, Yu Z, et al: Fetal membrane distention: I. Differentially expressed genes regulated by acute distention in amniotic epithelial (WISH) cells. Am J Obstet Gynecol 182:50, 2000

Nguyen H, Dubernard G, Aractingi S, et al: Feto-maternal cell trafficking: a transfer of pregnancy associated progenitor cells. Stem Cell Rev 2:111, 2006

Nicholson RC, King BR: Regulation of CRH gene expression in the placenta. Front Horm Res 27:246, 2001

Nikas G: Cell-surface morphological events relevant to human implantation. Hum Reprod 2:37, 2003

Nusken E, Wohlfarth M, Lippach G, et al: Reduced perinatal leptin availability may contribute to adverse metabolic programming in a rat model of

uteroplacental insufficiency. Endocrinology 157(5):1813, 2016

Ny T, Wahlberg P, Brandstrom IJ: Matrix remodeling in the ovary: regulation and functional role of the plasminogen activator and matrix metalloproteinase systems. Mol Cell Endocrinol 187:29, 2002

O'Sullivan CM, Liu SY, Karpinka JB, et al: Embryonic hatching enzyme strypsin/ISP1 is expressed with ISP2 in endometrial glands during implantation. Mol Reprod Dev 62:328, 2002

Odagiri E, Sherrell BJ, Mount CD, et al: Human placental immunoreactive corticotropin, lipotropin, and beta-endorphin: evidence for a common precursor. Proc Natl Acad Sci USA 76:2027, 1979

Parker CR Jr, Carr BR, Simpson ER, et al: Decline in the concentration of low-density lipoprotein-cholesterol in human fetal plasma near term. Metabolism 32:919, 1983

Parker CR Jr, Simpson ER, Bilheimer DW, et al: Inverse relation between low-density lipoprotein-cholesterol and dehydroisoandrosterone sulfate in human fetal plasma. Science 208:512, 1980

Patel N, Alsat E, Igout A, et al: Glucose inhibits human placental GH secretion, in vitro. J Clin Endocrinol Metab 80:1743, 1995

Peluso JJ: Non-genomic actions of progesterone in the normal and neoplastic mammalian ovary. Semin Reprod Med 25:198, 2007

Peng B, Zhu H, Klausen C, et al: GnRH regulates trophoblast invasion via RUNX2-mediated MMP2/9 expression. Mol Hum Reprod 22(2):119, 2016

Pereira de Sousa FL, Chaiwangyen W, Morales-Prieto DM, et al: Involvement of STAT1 in proliferation and invasiveness of trophoblastic cells. Reprod Biol 17(3):218, 2017

Petraglia F, Florio P, Benedetto C, et al: Urocortin stimulates placental adrenocorticotropin and prostaglandin release and myometrial contractility in vitro. J Clin Endocrinol Metab 84:1420, 1999

Petraglia F, Giardino L, Coukos G, et al: Corticotropin-releasing factor and parturition: plasma and amniotic fluid levels and placental binding sites. Obstet Gynecol 75:784, 1990

Petraglia F, Vaughan J, Vale W: Inhibin and activin modulate the release of gonadotropin-releasing hormone, human chorionic gonadotropin, and progesterone from cultured human placental cells. Proc Natl Acad Sci USA 86:5114, 1989

Petraglia F, Woodruff TK, Botticelli G, et al: Gonadotropin-releasing hormone, inhibin, and activin in human placenta: evidence for a common cellular localization. J Clin Endocrinol Metab 74:1184, 1992

Pijnenborg R: Trophoblast invasion. Reprod Med Rev 3:53, 1994

Pijnenborg R, Bland JM, Robertson WB, et al: Uteroplacental arterial changes related to interstitial trophoblast migration in early human pregnancy. Placenta 4:397, 1983

Piper KP, McLarnon A, Arrazi J, et al: Functional HY-specific CD8+ T cells are found in a high proportion of women following pregnancy with a male fetus. Biol Reprod 76:96, 2007

PrabhuDas M, Bonney E, Caron K, et al: Immune mechanisms at the maternal-fetal interface: perspective and challenges. Nat Immunol 16(4):328, 2015

Prakash A, Laird S, Tuckerman E, et al: Inhibin A and activin A may be used to predict pregnancy outcome in women with recurrent miscarriage. Fertil Steril 83(6):1758, 2005

Qin X, Chua PK, Ohira RH, et al: An autocrine/paracrine role of human decidual relaxin. II. Stromelysin-1 (MMP-3) and tissue inhibitor of matrix metalloproteinase-1 (TIMP-1). Biol Reprod 56:812, 1997a

Qin X, Garibay-Tupas J, Chua PK, et al: An autocrine/paracrine role of human decidual relaxin. I. Interstitial collagenase (matrix metalloproteinase-1) and tissue plasminogen activator. Biol Reprod 56:800, 1997b

Rahmati M, Petitbarat M, Dubanchet S, et al: Colony stimulating factors 1, 2, 3 and early pregnancy steps: from bench to bedside. J Reprod Immunol 109:1, 2015

Rainey WE, Carr BR, Wang ZN, et al: Gene profiling of human fetal and adult adrenals. J Endocrinol 171:209, 2001

Rajagopalan S, Long EO: Cellular senescence induced by CD158d reprograms natural killer cells to promote vascular remodeling. Proc Natl Acad Sci USA 109(50):20596, 2012

Ramsey EM, Donner MW: Placental Vasculature and Circulation. Philadelphia, Saunders, 1980

Reyes L, Wolfe B, Golos T: Hofbauer cells: placental macrophages of fetal origin. Results Probl Cell Differ 62:45, 2017

Richards JS: Genetics of ovulation. Semin Reprod Med 25(4):235, 2007

Riddick DH, Luciano AA, Kusmik WF, et al: Evidence for a nonpituitary source of amniotic fluid prolactin. Fertil Steril 31:35, 1979

Riley S, Walton J, Herlick J, et al: The localization and distribution of corticotropin-releasing hormone in the human placenta and fetal membranes throughout gestation. J Clin Endocrinol Metab 72:1001, 1991

Robidoux J, Simoneau L, St Pierre S, et al: Characterization of neuropeptide Y-mediated corticotropin-releasing factor synthesis and release from human placental trophoblasts. Endocrinology 141:2795, 2000

Robinson BG, Emanuel RL, Frim DM, et al: Glucocorticoid stimulates expression of corticotropin-releasing hormone gene in human placenta. Proc Natl Acad Sci USA 85:5244, 1988

Rogers PA, Donoghue JF, Walter LM, et al: Endometrial angiogenesis, vascular maturation, and lymphangiogenesis. Reprod Sci 16(2):147, 2009

Rosario FJ, Powell TL, Jansson T: Activation of placental insulin and mTOR signaling in a mouse model of maternal obesity associated with fetal overgrowth. Am J Physiol Regul Integr Comp Physiol 310(1):R87, 2016a

Rosario GX, Stewart CL: The multifaceted actions of leukaemia inhibitory factor in mediating uterine receptivity and embryo implantation. Am J Reprod Immunol 75(3):246, 2016b

Rowe T, King L, MacDonald PC, et al: Tissue inhibitor of metalloproteinase-1 and tissue inhibitor of metalloproteinase-2 expression in human amnion mesenchymal and epithelial cells. Am J Obstet Gynecol 176:915, 1997

Ruocco MG, Chaouat G, Florez L, et al: Regulatory T-cells in pregnancy: historical perspective, state of the art, and burning questions. Front Immunol 5:389, 2014

Ryan KJ: Biological aromatization of steroids. J Biol Chem 234:268, 1959a

Ryan KJ: Metabolism of C-16-oxygenated steroids by human placenta: the formation of estriol. J Biol Chem 234:2006, 1959b

Salido EC, Yen PH, Barajas L, et al: Steroid sulfatase expression in human placenta: immunocytochemistry and in situ hybridization study. J Clin Endocrinol Metab 70:1564, 1990

Saunders PT: Does estrogen receptor beta play a significant role in human reproduction? Trends Endocrinol Metab 16:222, 2005

Saxe A, Dean S, Gibson G, et al: Parathyroid hormone and parathyroid hormone-related peptide in venous umbilical cord blood of healthy neonates. J Perinat Med 25:288, 1997

Segaloff A, Sternberg W, Gaskill C: Effects of luteotrophic doses of chorionic gonadotropin in women. J Clin Endocrinol Metab 11:936, 1951

Short R: Steroids in the follicular fluid and the corpus luteum of the mare. A “two cell type” theory of ovarian steroid synthesis. J Endocrinol 24:59, 1962

Shozu M, Akasofu K, Harada T, et al: A new cause of female pseudohermaphroditism: placental aromatase deficiency. J Clin Endocrinol Metab 72:560, 1991

Siiteri PK, MacDonald PC: Placental estrogen biosynthesis during human pregnancy. J Clin Endocrinol Metab 26:751, 1966

Siiteri PK, MacDonald PC: The utilization of circulating dehydroisoandrosterone sulfate for estrogen synthesis during human pregnancy. Steroids 2:713, 1963

Siler-Khodr TM: Chorionic peptides. In McNellis D, Challis JRG, MacDonald PC, et al (eds): The Onset of Labor: Cellular and Integrative Mechanisms. Ithaca, Perinatology Press, 1988

Siler-Khodr TM, Khodr GS: Content of luteinizing hormone-releasing factor in the human placenta. Am J Obstet Gynecol 130:216, 1978

Siler-Khodr TM, Khodr GS: Dose response analysis of GnRH stimulation of hCG release from human term placenta. Biol Reprod 25:353, 1981

Siler-Khodr TM, Khodr GS, Valenzuela G: Immunoreactive gonadotropin-releasing hormone level in maternal circulation throughout pregnancy. Am J Obstet Gynecol 150:376, 1984

Simmonds CS, Karsenty G, Karaplis AD, et al: Parathyroid hormone regulates fetal-placental mineral homeostasis. J Bone Miner Res 25(3):594, 2010

Simoni MK, Jurado KA, Abrahams VM, et al: Zika virus infection of Hofbauer cells. Am J Reprod Immunol 77(2):1, 2017

Simpson ER: Genetic mutations resulting in loss of aromatase activity in humans and mice. J Soc Gynecol Investig 7:S18, 2000

Simpson ER, Burkhart MF: Acyl CoA:cholesterol acyl transferase activity in human placental microsomes: inhibition by progesterone. Arch Biochem Biophys 200:79, 1980

Simpson ER, Carr BR, Parker CR Jr, et al: The role of serum lipoproteins in steroidogenesis by the human fetal adrenal cortex. J Clin Endocrinol Metab 49:146, 1979

Sipos Pl, Rens W, Schlecht H, et al: Uterine vasculature remodeling in human pregnancy involves functional macrochimerism by endothelial colony forming cells of fetal origin. Stem Cells 31:1363, 2013

Soares MJ, Chakraborty D, Renaud SJ, et al: Regulatory pathways controlling the endovascular invasive trophoblast cell linear. J Reprod Dev 58(3):283, 2012

Song Y, Keelan J, France JT: Activin-A stimulates, while transforming growth factor beta 1 inhibits, chorionic gonadotrophin production and aromatase activity in cultures human placental trophoblasts. Placenta 17(8):603, 1996

Steele GL, Currie WD, Yuen BH, et al: Acute stimulation of human chorionic gonadotropin secretion by recombinant human activin-A in first trimester human trophoblast. Endocrinology 133:297, 1993

Stevens AM: Microchimeric cells in systemic lupus erythematosus: targets or innocent bystanders? Lupus 15:820, 2006

Sugino N, Kashida S, Karube-Harada A, et al: Expression of vascular endothelial growth factor (VEGF) and its receptors in human endometrium throughout the menstrual cycle and in early pregnancy. Reproduction 123:379, 2002

Thiruchelvam U, Dransfield I, Saunders PTK, et al: The importance of the macrophage within the human endometrium. J Leukoc Biol 93(2):217, 2013

Tomer Y, Huber GK, Davies TF: Human chorionic gonadotropin (hCG) interacts directly with recombinant human TSH receptors. J Clin Endocrinol Metab 74:1477, 1992

Trombly DJ, Woodruff TK, Mayo KE: Roles for transforming growth factor beta superfamily proteins in early folliculogenesis. Semin Reprod Med 27(1):14, 2009

Tsai SJ, Wu MH, Chen HM, et al: Fibroblast growth factor-9 is an endometrial stromal growth factor. Endocrinology 143:2715, 2002

Tsuruta E, Tada H, Tamaki H, et al: Pathogenic role of asialo human chorionic gonadotropin in gestational thyrotoxicosis. J Clin Endocrinol Metab 80:350, 1995

Tyson JE, Hwang P, Guyda H, et al: Studies of prolactin secretion in human pregnancy. Am J Obstet Gynecol 113:14, 1972

Vande Wiele RL, Bogumil J, Dyrenfurth I, et al: Mechanisms regulating the menstrual cycle in women. Recent Prog Horm Res 26:63, 1970

Vaskivuo TE, Ottander U, Oduwole O, et al: Role of apoptosis, apoptosis-related factors and 17 beta-hydroxysteroid dehydrogenases in human corpus luteum regression. Mol Cell Endocrinol 194(1–2):191, 2002

Vickers MH, Gilmour S, Gertler A, et al: 20-kDa placental hGH-V has diminished diabetogenic and lactogenic activities compared with 22-kDa hGH-N while retaining antilipogenic activity. Am J Physiol Endocrinol Metab 297(3):E629, 2009

Voltolini C, Battersby S, Novembri R, et al: Urocortin 2 role in placental and myometrial inflammatory mechanisms at parturition. Endocrinology 156(2):670, 2015

Wadhwa PD, Porto M, Garite TJ, et al: Maternal corticotropin-releasing hormone levels in the early third trimester predict length of gestation in human pregnancy. Am J Obstet Gynecol 179:1079, 1998

Walker WH, Fitzpatrick SL, Barrera-Saldana HA, et al: The human placental lactogen genes: structure, function, evolution and transcriptional regulation. Endocr Rev 12:316, 1991

Wallace EM, Swanston IA, McNeilly AS, et al: Second trimester screening for Down's syndrome using maternal serum dimeric inhibin A. Clin Endocrinol (Oxf) 44(1):17, 1996

Warren W, Silverman A: Cellular localization of corticotrophin releasing hormone in the human placenta, fetal membranes and decidua. Placenta 16:147, 1995

Weetman AP: The immunology of pregnancy. Thyroid 9:643, 1999

Wehmann RE, Nisula BC: Renal clearance rates of the subunits of human chorionic gonadotropin in man. J Clin Endocrinol Metab 50:674, 1980

Whittle WL, Gibb W, Challis JR: The characterization of human amnion epithelial and mesenchymal cells: the cellular expression, activity and glucocorticoid regulation of prostaglandin output. Placenta 21:394, 2000

Williams PJ, Searle RF, Robson SC, et al: Decidual leucocyte populations in early to late gestation normal human pregnancy. J Reprod Immunol

82(1):24, 2009

Winger EE, Reed JL: The multiple faces of the decidual natural killer cell. Am J Reprod Immunol 70:1, 2013

Wislocki GB, Dempsey EW: Electron microscopy of the human placenta. Anat Rec 123:133, 1955

Wolfahrt S, Kleine B, Rossmanith WG: Detection of gonadotrophin releasing hormone and its receptor mRNA in human placental trophoblasts using in situ reverse transcription-polymerase chain reaction. Mol Hum Reprod 4:999, 1998

Wu Z, Horgan CE, Carr O, et al: Biglycan and decorin differentially regulate signaling in the fetal membranes. Matrix Biol 34:266, 2014

Xie L, Sadovsky Y: The function of miR-519d in cell migration, invasion, and proliferation suggests a role in early placentation. Placenta 48:34, 2016

Yan C, Wang P, DeMayo J, et al: Synergistic roles of bone morphogenetic Protein 15 and growth differentiation Factor 9 in ovarian function. Mol Endocrinol 15:854, 2001

Yoshimura M, Pekary AE, Pang XP, et al: Thyrotropic activity of basic isoelectric forms of human chorionic gonadotropin extracted from hydatidiform mole tissues. J Clin Endocrinol Metab 78:862, 1994

Zhang M, Muralimanoharan S, Wortman AC, et al: Primate-specific miR-515 family members inhibit key genes in human trophoblast differentiation and are upregulated in preeclampsia. Proc Natl Acad Sci U S A 113(45):E7069, 2016