Part I

Conception and Conceptus Development

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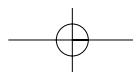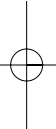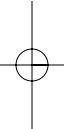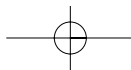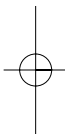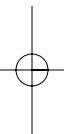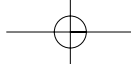
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Early conceptus growth and immunobiologic adaptations of pregnancy

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Reproduction will only be successful if a multitude of intricate sequences and interactions occur. This reproductive process begins with the formation of individual male and female gametes. Following gamete formation, a mechanism must be provided to ensure that these gametes attain close proximity to each other so fertilization may take place. After successful fertilization, the newly formed embryo must develop correctly and finally implant in a nourishing environment. Recently, there have been many advances in the understanding of these reproductive processes; however, it is beyond the scope of this chapter to provide detailed information on gamete formation, fertilization, and implantation. The interested reader is referred to several excellent texts for more specific information.[1,2] Rather, this chapter summarizes key normal developmental and physiologic events in early conceptus growth and immunobiologic adaptation of a pregnancy.

Gametogenesis

Gametogenesis is the maturational process that produces specialized gametes: the spermatozoon in the male and the oocyte in the female. Both cytoreduction and division prepare gametes for fertilization, which involves the union of male and female gametes. In order to maintain a constant chromosome number, the gametes undergo meiosis, a specialized form of cell division responsible for reducing the diploid number (46) of chromosomes to the haploid number (23).

At approximately 5 weeks of gestation, primitive germ cells migrate, presumably by way of ameboid movement from the yolk sac to the gonadal ridges. Following their migration, the germ cells are surrounded by somatic cells derived from the mesonephros forming the primary sex cords.[3]

In the first meiotic division, homologous chromosomes pair during prophase. In the pachytene stage of prophase, independent assortment and recombination of genetic material occurs among the gametes. Separation of the paired chromosomes occurs in anaphase, whereupon each new daughter cell

contains the haploid chromosome number or 23 double-structured chromosomes.

Shortly after the first division, the cell enters the second meiotic division. Each double-structured chromosome divides to form two separate chromosomes containing one chromatid. The resultant products include four daughter cells each containing the haploid number of chromosomes. Thus, one primary oocyte gives rise to four daughter cells, each receiving 22 autosomes and an X chromosome, and the primary spermatocyte gives rise to four daughter cells, each receiving 22 autosomes and either an X or a Y chromosome.
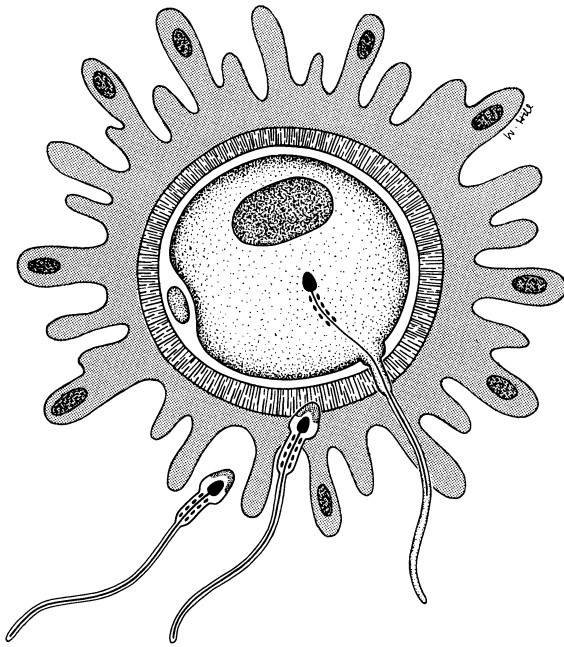
Fertilization

Embryonic development begins with the process of fertilization, the union of individual male and female gametes (Fig. 1.1). The fusion of two haploid cells, each bearing 22 autosomes and one sex chromosome, creates an offspring whose genetic makeup is different from that of both parents. Fertilization consists of a regulated sequence of interactions that will ultimately result in embryo development (Fig. 1.2).

Prior to any sperm–egg interaction, a requisite maturation of spermatozoa, termed capacitation, must occur.[4,5] The spermatozoa gain this ability during the transit through the female reproductive tract. Triggered exocytosis is the final consequence of capacitation.[6] The importance of capacitation has long been recognized, with the initial observation that capacitated sperm can readily penetrate the cumulus.[7] Capacitation is characterized by the acrosome reaction (AR) , the ability to bind to the zona pellucida (ZP), and the acquisition of hypermobility.

Spermatozoa must pass through an investment of cells and matrices, the cumulus, before any sperm–egg interaction may take place. The cumulus is composed of granulosa cells and a matrix consisting primarily of hyaluronic acid and proteins. Sperm capacitation and the hyperactivated motility seem to be important in the sperm's ability to penetrate the cumulus. Investigations have revealed that the sperm protein PH-20 is

Figure 1.1 Fertilization. A sperm is shown penetrating an oocyte. The spermatozoon must first undergo capacitation. Next, the sperm must penetrate the cumulus (the investment of cells and matrix surrounding the oocyte). After cumulus penetration, the sperm binds to the zona pellucida via specific receptors. The plasma membranes of the sperm and oocyte fuse. The sperm and tail of the sperm enter the oocyte, leaving the sperm's plasma membrane.

Completion of capacitation in the oviductal isthmus

↓

Admission into and through the cumulus matrix

↓

Primary binding to ZP3 on the sperm plasma membrane

↓

Triggering of acrosomal exocytosis

↓

Secondary binding to ZP2 using components exposed after the acrosomal reaction

↓

Autoactivation of proacrosin to acrosin, with attendant digestion through the ZP matrix

↓

Binding, followed by fusion between sperm and egg plasma membranes

Figure 1.2 Proposed sequence for mammalian gamete interaction. ZP, zona pellucida. (Adapted from ref. 35, with permission.)

also involved with cumulus matrix penetration.[8] Although PH-20 degrades hyaluronic acid and possesses similar protein properties to hyaluronidase, the exact role of this enzyme still remains uncertain.

The ZP is an acellular glycoprotein coat that covers and protects the ovum. The ZP is the last physical barrier that spermatozoa must pass before fertilization with the ovum. The initial interaction between the sperm and the oocyte ZP appears to be a receptor-mediated process. The ZP consists principally of three heavily glycosylated proteins: ZP1, ZP2, and ZP3.[9,10] Extensive studies, especially in the mouse, have revealed ZP2 and ZP3 function in sperm binding, whereas ZP1 serves a structural role.[5,11] Moreover, ZP3 has been demonstrated to be responsible for primary sperm binding (binding prior to the acrosome reaction) and triggers the acrosome reaction, while ZP2 is involved with secondary binding (binding with sperm following the acrosome reaction).[12,13]

The AR involves fusion between the sperm's plasma and acrosomal membrane with exocytosis of the enzyme contents of the acrosome. These enzymes, including hyaluronidase and acrosin, appear to play a role in ZP penetration. Furthermore, the AR changes the sperm head membranes in preparation for the eventual fusion of the inner acrosomal membrane with the

oocyte's plasma membrane. Acrosome-intact sperm are unable to fuse with oocytes.[14] Thus, the AR is an absolute prerequisite for sperm fusion with the oocyte membrane.

Once the ZP has been penetrated, the spermatozoon enters the perivitelline space at an angle and crosses quickly. The sperm then binds to the oocyte plasma membrane (oolemma) and soon the entire head enters the cytoplasm of the oocyte (ooplasm). Subsequently, there is fusion of the sperm and egg membranes with specific proteins mediating this process. One such fusion protein is fertilin (formerly called PH-30).[8,15] This sperm membrane protein appears to bind to the oolema via an integrin receptor-mediated mechanism.[8] Following fusion, several morphologic and biochemical events are initiated in the fertilized ovum.

Upon fusion of the egg and sperm membranes, there is a triggering of the cortical and zona reactions. As a result of the release of cortical granules in the oocyte, the oolema becomes impenetrable to spermatozoa. Furthermore, the ZP alters its structure, possibly due to ZP2 and ZP3 protein rearrangement, to prevent further sperm binding.[5] These are the primary blocking mechanisms to polyspermy.

Besides the cortical and zona reactions, a number of biochemical and molecular events are activated in the oocyte after sperm–egg fusion. Initially, there is a transient release of

intracellular calcium in a repeated oscillatory fashion.[16,17] These calcium pulses may be initiated by membrane depolarization and propagated through inositol triphosphate production. Consequently, the release of calcium induces exocytosis of the cortical granules. Eventually, these events will lead to initiation of the cell cycle and DNA synthesis.

Upon initiation, the oocyte will resume the second meiotic division that had been arrested at metaphase 2. One of the daughter cells will be extruded as the second polar body, while the other daughter cell, containing a haploid number of chromosomes, becomes the definitive oocyte. Restoration of the diploid number of chromosomes results from the addition of chromosomes from the sperm upon fertilization.

The female pronucleus is formed from the maternal chromosomes remaining in the oocyte. Meanwhile, the sperm head's chromatin decondenses, while enlarging the head in the ooplasm, forming the male pronucleus. The two pronuclei enlarge and migrate toward each other in the center of the fertilized egg. As the pronuclei move into close proximity, the nuclear membranes break down. Syngamy then begins as the chromosomes condense during the first cell division.

Preimplantation embryo

The initial phases of embryonic growth following fertilization are concerned with rapid cell division (Fig. 1.3). This initial increase in cell numbers is critical in establishing a sufficient number of cells in the embryo, which can then initiate differentiation. These cells are known as blastomeres. Beginning

with the first division, approximately 24–30 hours after fertilization, the blastomeres become smaller with successive divisions. Until the eight-cell stage, the cells are in a loosely arranged clump; however, following this cleavage stage, blastomeres begin merging into a coherent mass of cells marked by the formation of gap and tight junctions.[18,19] This process of compaction segregates inner cells from outer cells and represents the onset of embryonic differentiation. Approximately 3 days after fertilization, the berry-like mass of cells, termed the morula, enters the uterus.

The next event in embryo development is the formation of a fluid-filled cavity, the blastocele. With blastocyst formation, there is a partitioning of cells between an inner cell mass, the embryoblast, and an outer mass of cells, the trophectoderm. E-cadherin, a molecule involved with cell–cell binding, seems to be important for trophectoderm and blastocyst formation.[20] This polarization of blastomeres permits differentiation to proceed. Differentiation allows for the development of the three primitive tissue layers: the endoderm, mesoderm, and ectoderm. The primitive endoderm arises from a flattened layer of cells, the hypoblast, which lies on the surface of the inner cell mass and faces the blastocoele. Meanwhile, both the mesoderm and the ectoderm develop from the epiblast, the high columnar cell of the inner cell mass.

Until this stage in its growth, the blastocyst is still entirely surrounded by the ZP. The primary function of the ZP appears to be prevention of polyspermy. However, the ZP must be shed prior to embryo implantation to allow for the increasing cell mass and to enable contact between the embryo and the endometrium. This is achieved by hatching, where the embryo wiggles and squeezes out of this investment through a hole. In
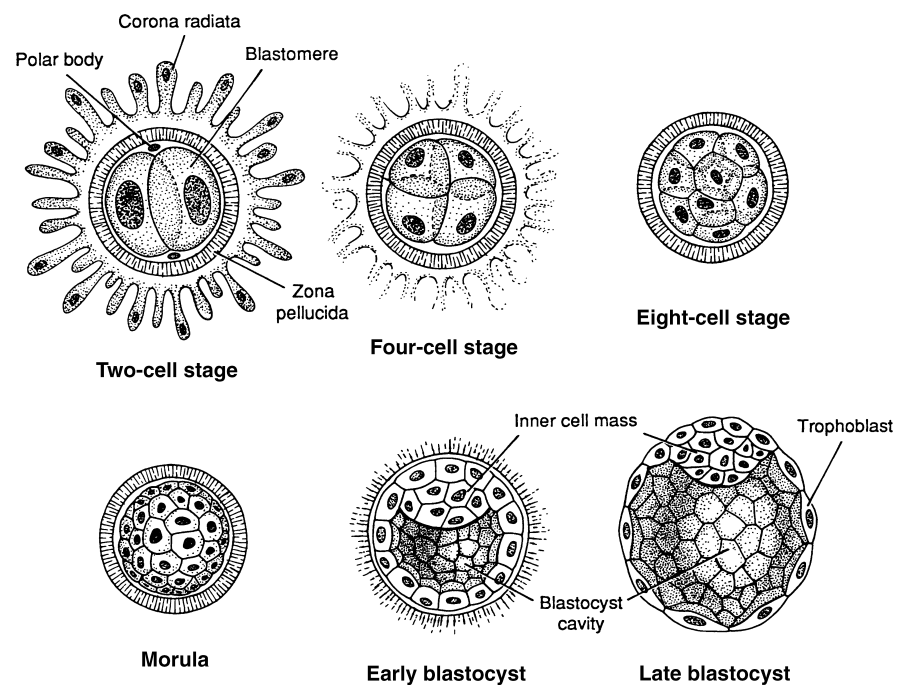
Figure 1.3 Cleavage and blastogenesis. Cleavage occurs in stages and results in the formation of blastomeres. The morula is composed of 12–16 blastomeres. The blastocyst forms when approximately 60 blastomeres are present. Note that the zona pellucida has disappeared by the late blastocyst stage. Until the zona pellucida is shed, the developing embryo essentially does not increase in size.

mice, the initial hole in the ZP is created by the enzyme trypsin.[21] In contrast, the exact mechanism in the human is still unknown, and human hatching has only been seen *in vitro*.[22]

After entering the uterus, the developing blastocyst floats inside the endometrial cavity for about 2–3 days. The embryo begins implantation approximately 6 days after fertilization, while the primitive germ layers develop between days 6 and 8. Following initial implantation, the embryo is completely imbedded within the endometrium by approximately 8–9 days after ovulation.

Intermediary metabolism in the developing embryo

Like all other cells, the developing embryo has nutritional requirements and possesses few nutrient stores, so it must depend on external sources. The metabolic requirements may vary depending on the particular embryonic stage of development. One requirement of particular interest is that pyruvate appears to be the major energy source for early embryo development, while glucose metabolism becomes activated in later cleavage stages. Besides pyruvate and glucose, there are many embryo nutrients and stimulants, including amino acids, intermediaries regulating calcium, and free radical scavengers, to name a few (see Fig. 1.4).

Molecular synthesis in the developing conceptus

The early conceptus exhibits a high level of metabolic activity and is capable of the synthesis and secretion of a number of macromolecules that have diverse effects on the success of implantation, placentation, and maintenance of pregnancy.

Among the earliest substances secreted by the preimplantation embryo is a soluble ether phospholipid, platelet activating factor (PAF). Correlation between the production of embryo-derived (ED)PAF and the pregnancy potential of embryos suggests that it may serve a fundamental role in the establishment of pregnancy.[23] Apparently, human embryos release variable amounts of PAF within 48 hours after fertilization.[24] Conclusive evidence for the essential role of PAF in the establishment of pregnancy was provided by Spinks and O'Neill, who used inhibitors of PAF activity *in vivo* to induce implantation failure in animals.[25]

Human chorionic gonadotropin (hCG) is a glycoprotein composed of one α and one β subunit with amino acid sequences similar to luteinizing hormone. It is produced by the early human trophoblast beginning about the eight-cell stage and is essential for the survival of the conceptus by stimulating progesterone production from the corpus luteum and thus preventing luteolysis and menstruation. In the human, implantation occurs on day 6 after ovulation, and hCG is first measurable on day 9 following ovulation.[26] The hCG production of human blastocysts *in vitro* has been correlated with their morphology and maturity, with the best embryos producing more hCG.[27]

Early pregnancy factor (EPF) has been described based on an alteration in lymphocytic reactivity in the lymphocyte rosette test, which was devised to assess the immunosuppressive characteristics of antilymphocyte serum *in vitro*.[28] Isolation of EPF in embryo growth media has been reported in several species. An immunosuppressive role has been implicated, possibly by modulating the maternal immune system.[29] Recently identified as part of a highly conserved heat shock family of molecules, EPF consists of an amino acid sequence with approximately 70% homology to chaperonin 10 and may be involved in protein binding.[30] EPF becomes positive in maternal serum as early as 24–48 hours after conception and therefore may be useful in the evaluation of early pregnancy failure.[31] Consequently, disorders of menstruation may be distinguished from early spontaneous abortion.

The human zygote produces a factor *in vitro* that is directly immunosuppressive.[32] Unlike the immunosuppressive actions of EPF or EDPAF, the actions of immunosuppressive factor (IF) are direct. The factor obtained from culture media of human embryos after *in vitro* fertilization suppresses mitogen-induced proliferation of peripheral lymphocytes, and those embryos producing the factor alone result in pregnancy. The presence of embryo-associated IFs at various stages of gestation may play a role in suppressing maternal cellular immune responses and prevent maternal rejection of the fetal allograft. Although IF was thought initially to derive from the developing embryo, recent evidence has localized IF to decidual cells.[33]

Although the mechanism has not been elucidated, histamine is thought to play a role in implantation of the blastocyst. Embryo-derived histamine releasing factor (EHRF) has been identified in culture medium used to grow developing embryos.[34] Both calcium and temperature dependent, EHRF induces histamine release from sensitized basal cells. Although the role of this factor remains to be clarified, EHRF could represent a message sent by the embryo to the mother to induce histamine release at the time of implantation.

Cytokines and growth factors regulating implantation

A critical stage in development involves embryonic implantation, a continual synchrony between the embryo itself and a complex series of molecular and cellular events induced in the uterus by estrogen and progesterone. Much of this maternal environment/embryonic "talk" is mediated in an autocrine/paracrine manner by cytokines and growth factors produced by both the embryo and the uterus. Although there exists a myriad of information concerning cytokine and growth factor involvement with implantation, the complete details of this mechanism are still incomplete. For a more comprehensive
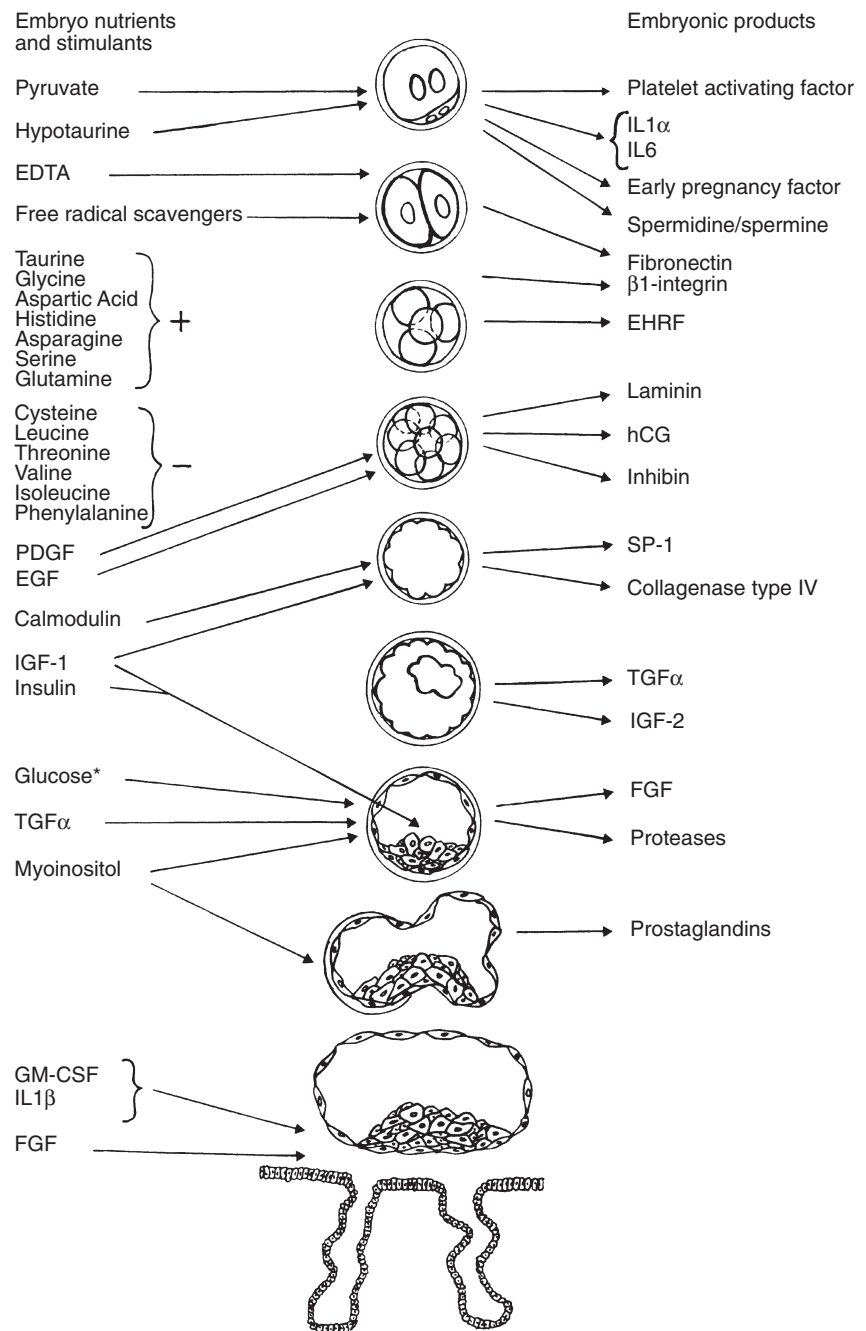
Figure 1.4 Embryonic nutrients and secreted products. EDTA, ethylenediaminetetraacetic acid; EGF, epidermal growth factor; EHRF, embryo-derived histamine-releasing factor; FGF, fibroblast growth factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; hCG, human chorionic gonadotropin; IGF, insulin-like growth factor; IL, interleukin; PDGF, platelet-derived growth factor; TGF, transforming growth factor. (From ref. 55, with permission.)

review, the interested reader is referred to several reviews.[35,36] At least three cytokines, colony-stimulating factor 1 (CSF-1), leukemia inhibitory factor (LIF) and interleukin 1 (IL-1) appear to be involved in implantation.[37]

Apposition and adhesion of the embryo to the endometrium

The blastocyst lies unattached in the uterine endometrial cavity for approximately 2 days before implantation. Implan-

tation begins as the embryo becomes closely apposed to the endometrial epithelium (Fig. 1.5). The initial contact is made via the polar trophectoderm. Apposition seems to allow the complementary binding proteins of the embryo and endometrial epithelium to function effectively during implantation by the interdigitating of epithelial cells and trophoblast with microvilli.

The adherence of the blastocyst to the endometrial epithelium appears to be mediated through ligand–receptor complexes. The expression of specific adhesion molecules, such as integrins, in the embryo and specific substrates and receptors,

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A

Endometrial
glands

Endometrial
capillaries

Endometrial
epithelium

Inner cell
mass

Embryonic pole

Blastocyst cavity

Trophoblast

B

Syncytiotrophoblast

Cytotrophoblast

C

Syncytiotrophoblast

Amniotic
cavity

Embryonic
epiblast

Exocoelomic
membrane

Cytotrophoblast

Embryonic
hypoblast

D

Amnion

Embryonic
disc

Primary
yolk sac

Extraembryonic
mesoderm

Maternal blood
in lacunae

Figure 1.5 Implantation. (A) After floating free for 2 days, the polar trophectoderm of the embryo apposes the endometrial epithelium. (B) Penetration begins with rapid proliferation and differentiation into two cell types, the cytotrophoblast and the syncytiotrophoblast. The syncytiotrophoblast, a multinucleated mass of cells with no cell boundaries, extends through the endometrial epithelium to penetrate the stroma. (C) The inner cell mass differentiates into the epiblast, which gives rise to the mesoderm and ectoderm, and the hypoblast, which gives rise to the endoderm. (D) The embryo becomes completely embedded 7–13 days after ovulation.

such as laminin, fibronectins, and collagen IV, in the uterine epithelium and decidua appears to be involved with these ligand–receptor complexes. After adhering to the uterine epithelium, the blastocyst will begin penetrating through the basement membrane and into the uterine stroma.

Penetration of the epithelium

Immediately following adhesion, the blastocyst begins penetration into the endometrial epithelium and stroma (Fig. 1.5). For trophoblast cells to invade, they have to degrade and remodel the epithelium and stroma. Thus, embryos must produce specific molecules and other enzymes to assist in their penetration. A delicate coordination must exist, however, between the invading embryo and the underlying endometrium to prevent excessive penetration and yet provide adequate invasiveness.

The enzymes and molecules implicated in implantation include the proteases, proteinases, and their inhibitors, which are all involved with degradation of the extracellular matrix. There is a high degree of tissue reorganization that occurs during implantation. At present, the significance of these substances is not fully understood, although their importance in implantation is paramount. Clearly, further studies are needed to elucidate whether one or more of these systems are involved in embryo penetration or if they are redundant systems for "back up" in case one of the systems should become ineffective.

The early human trophoblast

The blastocyst attaches to the endometrial epithelium at the embryonic pole 6 days after fertilization (Fig. 1.5). After the trophoblast has attached to the endometrial epithelium, rapid cellular proliferation occurs, and the trophoblast differentiates into two layers consisting of the inner cytotrophoblast and an outer syncytiotrophoblast, a multinucleated mass without cellular boundaries. Syncytial trophoblast processes extend through the endometrial epithelium to invade the endometrial stroma. Stromal cells surrounding the implantation site become laden with lipids and glycogen, become polyhedral in shape, and are referred to as decidual cells. These decidual cells degenerate in the region of the invading syncytiotrophoblast and provide nutrition to the developing embryo. The blastocyst superficially implants in the stratum compactum of the endometrium by the end of the first week. The trophoblast then invades the surrounding myometrium as the blastocyst becomes completely imbedded in the decidua. Capillary connections are formed as the trophoblast invades, and the blood supply to the developing fetus is established through which it will obtain its support until delivery occurs.

Immunobiologic adaptations of pregnancy

The primary role of the immune system is to protect the body from invasion by foreign organisms and their toxic products. This requires an ability to discriminate between self and nonself antigens, so that immune destruction can be targeted against the invading organism and not against the animal's own tissues. In pregnancy, the antigenically foreign fetus grows in its mother for 9 months, unharmed by her immune system. Clearly, immune adaptations must occur in pregnancy that are central to the survival of the fetus while maintaining the mother's ability to fight infection.

The maternal–fetal interface

Trophoblast

The fetus itself does not come into direct contact with maternal tissue. The trophoblast of the placenta and fetal membranes forms the interface between mother and fetus. Two areas of contact between mother and fetus are established: (1) a large surface area formed by the syncytiotrophoblast of the chorionic villi that is bathed by maternal blood; and (2), within the deciduas, extravillous trophoblast (mostly cytotrophoblast but with some syncytial elements) that mingles directly with maternal tissues.

Fetal–maternal cell traffic

The villous syncytiotrophoblast, adjacent to blood, and the nonvillous cytotrophoblast, in contact with maternal deciduas, are the main areas where maternal lymphocytes might be sensitized to trophoblasts. However, the interface between mother and fetus is extended by the traffic of fetal cells into the maternal circulation, carrying fetal antigens to other parts of the maternal immune system, where priming responses could also occur (Table 1.1).

Trophoblast deportation

It has been known for many years that trophoblast cells enter the maternal circulation.[38] There are two ways in which this might happen. First, trophoblast "buds" (called syncytial sprouts) often form on the syncytiotrophoblast surface and

Table 1.1 Contact between maternal and fetal tissues.

Local	Syncytiotrophoblast lining intervillous space
	Cytotrophoblast in decidua
Systemic	Fetal red and white cells entering maternal blood
	Trophoblast deportation

may break free and enter the maternal blood. This disruption of the syncytiotrophoblast could also lead to the underlying villous cytotrophoblast entering the mother's blood. Alternatively, the endovascular cytotrophoblast that lines the spiral arteries may be carried away into the bloodstream. There is evidence for both multinucleate (syncytiotrophoblast) and mononuclear (cytotrophoblast) cells entering the maternal uterine vein,[39] but it is not yet established whether the mononuclear cells are villous or extravillous cytotrophoblasts in origin. It is also a matter of great controversy whether trophoblasts enter the peripheral circulation to a major extent in pregnancy,[40] or whether they become trapped in the lungs.[41]

Traffic of fetal blood cells

Direct contact of fetal (as opposed to placental) cells with maternal cells can come about only by the passage of fetal blood into the maternal circulation. There is now good evidence that fetal nucleated erythrocytes can enter the maternal blood in early pregnancy,[42] and it must be assumed that fetal leukocytes will enter at the same time.[43] Therefore, it appears that more cells traverse the placental barrier as the fetus and the placenta grow.[44] Their presence is presumed to result from fetal–maternal hemorrhage, although the mechanism by which this occurs has yet to be defined.

Maternal immune cells in decidua

The decidua is the tissue in which immune recognition of trophoblasts is most likely to occur. Immunohistologic and flow cytometric studies of the first-trimester pregnancy decidua into which trophoblast invades have shown that it is composed predominantly of immune cells.[45] Approximately 10% of the stromal cells are T lymphocytes (although there are virtually no B cells) and 20% are macrophages;[46] these two cell types are essential for cell-mediated graft rejection responses. However, the main immune cell population is large granular lymphocytes or natural killer (NK) cells, comprising 45% of the decidual cells.[47] Immunohistologic studies show that the extravillous cytotrophoblast is in close contact with these immune cells, which raises the question as to how the trophoblast avoids recognition and rejection.

Maternal immune responses to trophoblast

Expression of major histocompatibility complex (MHC) antigens by trophoblast

The way in which the mother's immune system responds to trophoblast cells will depend on which, if any, MHC antigens they express; therefore, this has been an area of intense study. Studies using monoclonal antibodies that recognized all forms of class I antigens [human leukocyte antigen (HLA)-A, -B, and

Table 1.2 MHC expression in human development.

	Class I MHC		Class II MHC
	HLA-G	HLA-A, -B, -C	HLA-DR, -DP, -DQ
Oocyte	–	–	–
Sperm	–	–	–
Blastocyst	+	?	?
Syncytiotrophoblast	–	–	–
Villous cytotrophoblast	–	–	–
Extravillous cytotrophoblast	+	–	–
Fetal tissue	–	+	+

MHC, major histocompatibility complex; –, antigen absent; +, antigen present; ?, not yet known.

-C] revealed that, although the syncytiotrophoblast and underlying villous cytotrophoblast were negative for class I, the invasive extravillous cytotrophoblast in the placental bed and the amniochorion strongly expressed this antigen.[48] Subsequent biochemical[49,50] and molecular analyses[51] have shown that the trophoblast class I antigen is in fact HLA-G. HLA-G differs from HLA-A, -B, and -C in that it is nonpolymorphic and has a lower molecular weight. The latter characteristic arises from a termination codon in exon 6, resulting in the transcription of a protein with a truncated cytoplasmic tail.[52]

Polyclonal antibody studies have confirmed that HLA-G protein is expressed only by extravillous cytotrophoblast[53] (Table 1.2). Neither oocytes[54] nor sperm express surface class I or class II antigens, although sperm are reported to express mRNA for both HLA-B and -G.[55] Similarly, oocytes appear to be negative for both class I and class II antigens. Cleavage-stage embryos and blastocysts were also thought to be negative for class I,[56] but there is no evidence that a proportion of blastocysts express both HLA-G mRNA and protein, which may be associated with more rapid cleavage rates.[57] Thus, expression of HLA-G at this stage could be vital to protect the embryo as it implants into the decidua.

Immunoregulatory role of HLA-G

Soluble class I HLA molecules are known to be shed into the serum of patients with HLA-mismatched organ grafts.[58] These donor-derived, soluble, class I antigens are believed to prolong graft survival by inhibiting the activity of alloreactive cytotoxic lymphocytes.[59] This may occur through their binding to the T-cell receptor or its coreceptor, CD8, which induces apoptosis of the cytotoxic T cell.[60] It has been proposed that soluble HLA-G may likewise be shed from the surface of the trophoblast and may eliminate maternal cytotoxic T cells by a similar mechanism.[61] In support of the hypothesis, evidence for a soluble HLA-G molecule has been obtained at both the

Table 1.3 Properties and functions of HLA-G.

Protein expression restricted to extravillous cytotrophoblast
Exists in both membrane-bound and soluble forms
Heavy chain (40-kDa) has truncated cytoplasmic tail
May have limited polymorphism or is nonpolymorphic
Forms class I complexes with β_2-microglobulin and antigenic peptides
Expression is associated with TAP1
Appears not to stimulate maternal T-cell responses
Downregulates NK cell-mediated cytotoxicity

NK cell, natural killer cell; TAP1, transporter associated with peptide presentation.

molecular[62] and the protein level,[50] and other studies have shown that HLA-G binds to CD8.[63]

HLA-G expression may also serve a protective role for trophoblasts. HLA-G inhibits the proliferation of CD4+ T lymphocytes[64] and decreases decidual cell production of interferon (IFN)-γ and tumor necrosis factor (TNF)-α.[65] Addition of HLA-G to mixed lymphocyte cultures increases the production of IL-10 and decreases IFN-γ and TNF-α production causing a shift from a Th1 to a Th2 phenotype.[66]

Protection against NK cell attack

It might seem that, in evolutionary terms, it would be simpler for the trophoblast not to express class I MHC and thereby avoid immune recognition. However, a major threat to trophoblast invading the decidua is presented by the large granular lymphocytes (NK cells). NK cells preferentially kill target cells that lack class I MHC. The presence of class I antigens on the cell surface is thought to be essential for protection from NK cell-mediated attack. Experiments using cell lines have shown that variants with low levels of class I expression are highly susceptible to NK lysis,[67] but that transfection with both classical class I and HLA-G genes can confer protection.[61,68] The expression of HLA-G may therefore be essential to protect extravillous cytotrophoblast from decidual NK cells.[69,70] Thus, HLA-G may serve a dual role in protecting trophoblast from both cytotoxic T cells and NK cells.

The properties and possible functions of HLA-G are summarized in Table 1.3.

Maternal immune responses to trafficking cells

Fetal leukocytes

In the placenta, class I antigen expression occurs in the mesenchyme of the chorionic villi as early as 2.5 weeks, although it is sporadic and weak. Class II-positive cells are found in the placenta by 14 weeks' gestation.[71] In the fetus itself, class I- and class II-positive cells have been found in the thymic epithelium at 7 weeks' gestation.[72] Thus, if fetal leukocytes enter the maternal circulation, they could potentially stimulate maternal immune responses.

Antibody responses

Antifetal (paternal) HLA alloantibodies can develop during a first pregnancy,[73] and may occur after an abortion,[74] which indicates that immunization is not necessarily the result of events at delivery, but usually develops after 28 weeks, with the incidence increasing with parity.[75] These antibodies do not develop in all pregnancies. The rate is approximately 15% of women in their first pregnancies and never more than approximately 60% among multiparous women.[76] Antibodies may develop against both class I and class II antigens.[77]

None of these antifetal antibodies appears to cause harm to the fetus, probably because they cannot bind to the syncytiotrophoblast, given that it does not express MHC antigens. This would be sufficient protection were it not for the placenta's role in the transfer of immunoglobulins from the maternal to the fetal circulation – a process by which the fetus acquires immunity from infection in the perinatal period. Fc receptors on the surface of the syncytiotrophoblast bind free immunoglobulin G (IgG) molecules and transport them to the villous stroma, where they enter the fetal circulation. Only IgG is transported; antibodies of other classes remain in the maternal blood. However, antibodies to fetal (paternal) HLA appear to be effectively filtered out by binding HLA antigens on cells in the villous stroma. IgG that is aggregated or complexed with antigen is removed by Fc receptor-bearing macrophages.[78] This illustrates the concept of the placental "sponge." Thus, only maternal IgG antibodies to antigens not represented in placental tissues escape the "sponge" and reach the fetal circulation.[79]

Cell-mediated responses

If the mother can develop antibodies to fetal HLA antigens, it would be expected that she can also develop cell-mediated immunity because T- and B-cell sensitization to fetal HLA should occur together. It is therefore surprising that there is only sporadic evidence for T-cell sensitization, as judged by the detection of a secondary maternal–paternal (fetal) mixed lymphocyte reaction or paternal (fetal)-specific cytotoxic T cells.[80]

A search for maternal cytotoxic T cells against paternal and unrelated control target cells at term found clear evidence for their presence in only 2 of 20 pregnant women.[81] In a further series of experiments, no sensitization to paternal HLA was seen in 25 normal first-trimester pregnancies.[82] Even when cytotoxic T cells were found, they did not appear to harm the fetus because these women had normal pregnancies. This implies that cytotoxic T cells cannot cross the placental barrier to gain access to the fetus.

Table 1.4 Maternal immune responses to fetal cells.

	Antibody response	Cell-mediated response
Fetal leukocytes	+	+/–
Trophoblast	+/– (?)	–

+, response; –, no response; (?), conflicting evidence.

Table 1.5 Alterations in maternal cellular immunity during pregnancy.

Component	Alteration in pregnancy	Reference
B-cell numbers	No change	102, 103
T-cell numbers	No change	104, 105, 106
T-cell function	No change	107
	Decreased	108, 109
NK-cell function	Decreased	110, 111

Immunoregulation

From the discussion above, it is clear that there is a paradox in pregnancy in that, although the mother's ability to produce antibodies is apparently normal, her ability to mount cell-mediated immune responses is weakened (Table 1.4). This concept is supported by clinical observations that pregnant women, although not grossly immunocompromised, are more susceptible to diseases that are normally dealt with by cell-mediated immune responses. Certain viral infections, such as hepatitis, herpes simples, and Epstein–Barr virus, are more common in pregnancy.[83] Diseases caused by intracellular pathogens (e.g., leprosy, tuberculosis, malaria, toxoplasmosis, and coccidioidomycosis) appear to be exacerbated by pregnancy. Furthermore, approximately 70% of women with rheumatoid arthritis (caused by cytotoxic T cells in the joints) experience a temporary remission of their symptoms during gestation, whereas systemic lupus erythematosus (caused by autoantibodies) tends to get worse during pregnancy.[84]

Many investigators have attempted to characterize the maternal immune response by determining immune cell subsets and immune cell function during pregnancy. In general, immune function is similar in pregnant and nonpregnant women (Table 1.5). Taken together, there is no clear trend toward either the enhancement or the suppression of immune function during pregnancy.

Immunoregulatory factors

Placental suppressor factors
The placenta itself can release factors that suppress T-cell and NK-cell activity.[85] Microvillous preparations of syncytiotrophoblast and culture supernatants from placental cells and choriocarcinoma cell lines[86,87] nonspecifically suppress mitogen responsiveness and allogenetically stimulated lymphocytes in the mixed lymphocyte reaction along with the cytolytic activity of cytotoxic T cells and NK-cell activity.[88] Suppressive activity may appear very early in gestation, given that animal[89] and human preimplantation embryos have been reported to produce inhibitory factors within 24 hours of fertilization.[90]

Decidual suppressor factors
Suppressive factors released by the placenta into the blood may inhibit lymphocyte responses systematically, but other mechanisms may be involved locally to prevent alloimmune recognition of extravillous cytotrophoblast that invades the decidua. Suppression of cell-mediated responsiveness *in vitro* by cell populations[91] from first-trimester human decidua has also been demonstrated. Decidual cells secrete various proteins that might mediate these suppressive activities. Transforming growth factor β, a cytokine that strongly inhibits proliferation of B cells and T cells and the cytolytic activity of NK cells, has been localized to the large granular lymphocytes in the human decidua.[92]

Cytokines and pregnancy

The strongest candidates for the suppressor factors derived from the placenta and decidua are cytokines. It has been proposed that the maternal immune changes in pregnancy are brought about by a shift in the balance of cytokines that favors antibody production and depresses the potentially harmful cell-mediated immune responses.

Type I and type 2 cytokines and the immune response
It has become apparent that antibody production and cell-mediated responses are controlled through two distinct populations of CD4+ Th cells.[93] Type 1 CD4+ Th cells (Th1) control cell-mediated responses by secreting cytokines such as IL-2, TFN-β, and IFN-γ, which stimulate cytotoxic T cells and NK cells (Th1 response). Type 2 CD4+ Th cells (Th2) produce IL-4, which stimulates IgE and IgG antibody production by B cells (Th2 response) (Fig. 1.6A). These two systems are also interactive in that IFN-γ produced by T1 cells inhibits B-cell development induced by Th2 cells, and Th2 cells in turn produce IL-10, which inhibits cytokine synthesis by Th1 cells (Fig. 1.6B). Thus, Th1 and Th2 cytokines are mutually inhibitory but, in the normal state, they are in balance, allowing both forms of immune response to coexist. However, a deviation in the pattern of cytokine production could lead to one type of response being favored over the other.
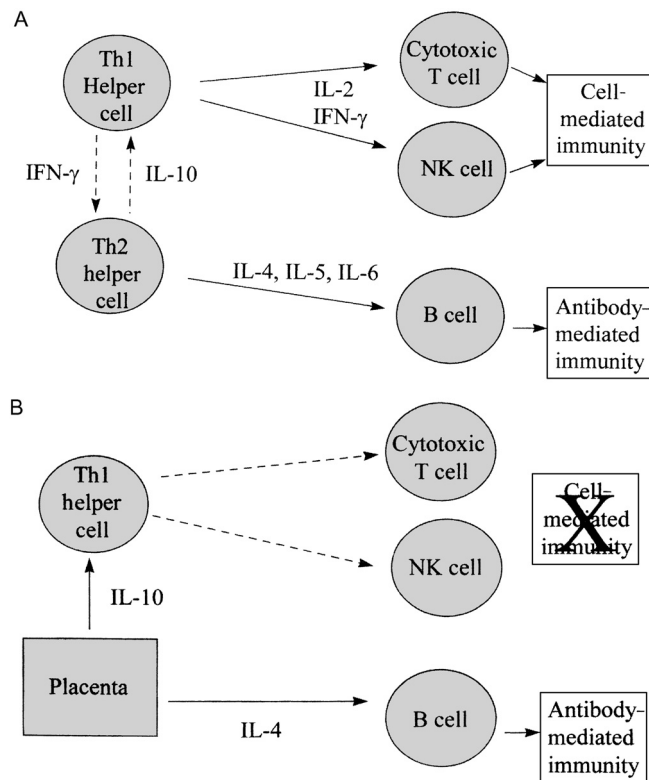
Figure 1.6 (A) Th1 and Th2 cytokines in immune responses. (B) Th1 and Th2 cytokines in pregnancy. IFN, interferon; IL, interleukin; NK, natural killer; Th1, type 1 T helper cells; Th2, type 2 T helper cells.

Type 1 and type 2 cytokines in pregnancy

In pregnancy, it is proposed that there is a shift away from Th1 responses and toward Th2 responses.[94] The cause of this shift is thought to be the production of Th2 cytokines by the placenta (Fig. 1.6B). Thus, excess IL-4 released from the placenta would stimulate maternal antibody responses. At the same time, excess IL-10 production would inhibit Th1 cells, leading to the suppression of cytotoxic T cells and NK-cell activity, which has been observed.

Experimental evidence for this hypothesis is largely confined to the mouse. Several groups have demonstrated that production of Th2 cytokines by tissues at the maternal–fetal interface[95,96] and injection of Th1 cytokines TNF-α, IFN-γ, and IL-2 into pregnant mice can increase fetal resorption rates and inhibit mouse embryo development and implantation *in vitro*.[97] So far, evidence in the human is restricted to localization studies showing that IL-4 is present in the syncytiotrophoblast, the cytotrophoblast of the fetal membranes, and decidual macrophages,[98] and that IL-10 is secreted by HLA-G-positive cytotrophoblast.[99] In contrast, IL-10 knockout mice[100] and IL-10, IL-4 double knockouts[101] have normal pregnancies. Thus, the immunologic relationship between mother and fetus may be more complex than originally thought.

Immune circuit

It is clear form the foregoing discussion that, in normal pregnancy, fetal growth progresses side by side with the development of a number of immune mechanisms that function at several levels. These can be summarized by constructing an immune circuit (Fig. 1.7A). The first stage in this circuit is the exposure of the maternal immune system to both fetal trophoblast and leukocytes. This could potentially lead to immune recognition and the development of cell-mediated and antibody responses to fetal antigens, which in turn would lead to rejection of the fetus (placenta). However, this circuit is broken at several stages (Fig. 1.7B). First, on the basis of current evidence, the maternal immune system does not recognize the trophoblast because it either fails to express HLA or expresses HLA-G. Second, although fetal leukocytes can be recognized by maternal immune cells, only antibody responses occur because the placenta's production of Th2 cytokines downregulates cell-mediated immunity. Finally, the production of antipaternal antibodies is not harmful because the placenta filters out these antibodies before they reach the fetal circulation. Thus, it is the combination of these many immune adaptations of pregnancy that ensure the success of the fetus.

A

B
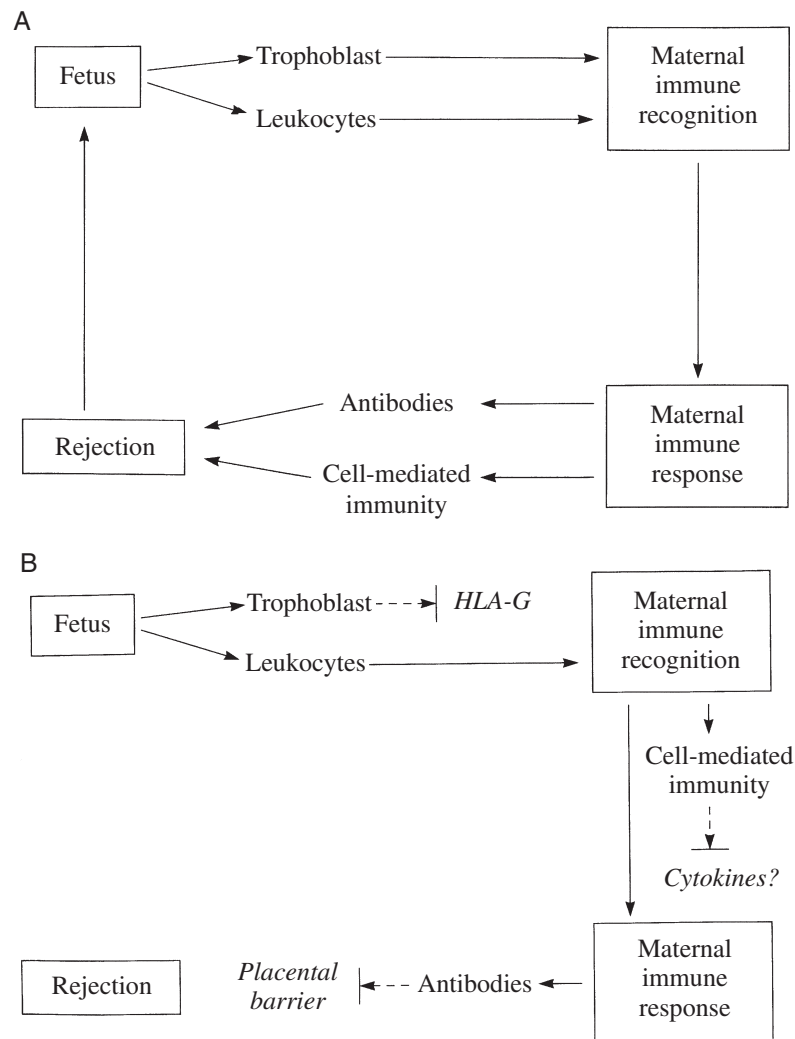
Figure 1.7 (A) Immune responses in pregnancy that could lead to rejection of the fetus. (B) Immuno-regulatory mechanisms in pregnancy that prevent the rejection of the fetus.

Key points

1 During meiosis, the primary oocyte gives rise to four daughter cells, each receiving 22 autosomes and an X chromosome. The primary spermatocyte also gives rise to four daughter cells, each receiving 22 autosomes and either an X or a Y chromosome.

2 Prior to sperm–egg interaction, capacitation of the spermatozoa must occur.

3 Capacitation is characterized by the acrosome reaction, fusion between the sperm's plasma and acrosomal membrane with exocytosis of the enzyme contents.

4 The zona pellucida is an acellular glycoprotein coat covering the ovum and consists of three principal proteins: ZP1, ZP2, and ZP3.

5 Upon fusion of the egg and sperm membranes, the cortical and zona reactions are triggered.

6 After egg–sperm fusion, the oocyte will resume the second meiotic division and extrude the second polar body.

7 The morula enters the uterus 3 days after fertilization and floats inside the endometrial cavity for 2–3 days. The embryo begins implantation approximately 6 days after fertilization.

8 Human chorionic gonadotrophin is a glycoprotein produced by the early conceptus and is essential in stimulating the corpus luteum to produce progesterone.

9 Three cytokines appear to be involved in implantation, colony-stimulating factor 1, leukemia inhibitory factor, and interleukin 1.

10 The adherence of the blastocyst to the endometrial epithelium is mediated through ligand–receptor complexes.

11 HLA-G protein is expressed only by extravillous cytotrophoblast.

12 Fetal nucleated erythrocytes and leukocytes can enter the maternal blood in early pregnancy.

13 First-trimester pregnancy decidua is composed predominantly of immune cells. Approximately 10% of the stromal cells are T lymphocytes, 20% are macrophages, and the main immune cell population is large granular lymphocytes or NK cells, comprising 45% of the decidual cells.

14 HLA-G inhibits the proliferation of CD4+ T lymphocytes and decreases decidual cell production of IFN-γ and TNF-α.

15 HLA-G may serve a dual role in protecting trophoblast from both cytotoxic T cells and NK cells.

16 In the placenta, class I antigen expression occurs in the mesenchyme of the chorionic villi as early as 2.5 weeks; class II-positive cells are found in the placenta by 14 weeks' gestation.

17 There is no clear trend toward either the enhancement or the suppression of immune function during pregnancy.

18 The placenta can release factors that suppress T-cell and NK-cell activity.

19 Type 1 CD4+ Th cells (Th1) control cell-mediated responses by secreting cytokines such as IL-2, TFN-β, and IFN-γ, which stimulate cytotoxic T cells and NK cells (Th1 response).

20 Type 2 CD4+ Th cells (Th2) produce IL-4, which stimulates IgE and IgG antibody production by B cells (Th2 response).

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