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Development, Function, and Pathology of the Placenta

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KEY POINTS

- The placenta is the first organ to form in mammals and is required for establishment of a maternal–fetal vascular interface capable of supplying the bioenergetic needs of the developing conceptus.
- Multiple placental cell types engage in highly varied functions, from attachment, invasion, and vascular remodeling to cell fusion, hormone production, and nutrient transport.
- Multiple mechanisms allow transport of waste and nutrients across the placenta, including diffusion, transporter protein-mediated (facilitated diffusion and active transporters), and receptor-mediated mechanisms.
- The placenta is not an inert transport interface. It consumes 40%–60% of the oxygen and glucose delivered to the uterus at term. Thus conditions that alter placental metabolism can indirectly affect nutrient transport to the fetus.
- Maternal and fetal health alter placental function, which in turn influences fetal adaptations and contributes to in utero fetal programming.
- Abnormal placentation and placental infections can lead to preeclampsia (PE), fetal growth retardation, or preterm birth, which can have lifelong bearing on health.
- In the United States, iatrogenic delivery is responsible for almost half the births that occur between 28 and 37 weeks of gestation, primarily caused by placental pathologies such as PE or intrauterine growth restriction.
- Efforts to standardize placental examination after delivery are in progress so that connections between specific placental problems and poor outcomes can be better defined. In parallel, new advanced imaging techniques and biomarkers for placental function are being developed.

The placenta is a remarkable organ. Its brief existence enables the mammalian fetus to survive and thrive within the otherwise inhospitable confines of the intrauterine environment. To accomplish this, the placenta plays a range of roles, from anchoring the conceptus and preventing its rejection by the maternal immune system to enabling the transport of nutrients and wastes between the mother and the embryo/fetus. As with all organs, multiple specialized cell types derived from lineage-committed precursors are responsible for these functions. Genetic, epigenetic, and physiologic cues direct placental development across gestational stages (Maltepe et al., 2010). Impairments in

these processes due to intrinsic or extrinsic insults can lead to placental dysfunction and result in long-lasting increases in disease susceptibility, a process known as fetal programming (Murphy et al., 2006; Eichenwald and Stark, 2008). Both preterm and term infants are at risk from poor placental function, particularly those that are extremely low birth weight (<1 kg). Preterm infants in particular may suffer from placental dysfunction in utero followed by early loss of placental support, including nutrition, hormones, and immune protection. Preterm delivery rates continue to rise while the survival of preterm infants has increased due to numerous advances in medical management and technology (Saigal and Doyle, 2008). This convergence has generated an expanded population of patients admitted to and graduating from intensive care nurseries. Not only are these infants more likely to develop complications such as bronchopulmonary dysplasia, failure to thrive, pulmonary hypertension, cerebral palsy, and blindness, but they are also more likely to develop chronic adult ailments such as diabetes and heart disease. Although further improvements in neonatal care are critical for diminishing the long-term consequences of prematurity, prevention or delay of preterm delivery will have the greatest healthcare impact for this at-risk population. A better understanding of the most common placental pathologies is therefore critically important for advancing maternal, fetal, and adult medicine.

Placental Origin

The mammalian lineage began approximately 200 million years ago with monotremes, or egg-laying mammals, e.g., the echidna and platypus. Echidnas lay their eggs into an egg pouch, whereas platypuses lay their eggs into a burrow, where the mother curls around them to provide them with warmth and protection. Lacking a true placenta, these embryos rely on a yolk sac to provide them with nourishment. The duck-billed platypus, on the other hand, develops a primitive allantoic vitelline placenta from trophoblast (TE)-like cells called vitellocytes. These support the embryo to the 19-somite stage, indicating that the molecular mechanisms enabling segregation of extraembryonic TE from embryonic tissues may have predated evolution of a true placenta (Hughes and Hall, 1998; Selwood and Johnson, 2006). Marsupial embryos also have yolk sacs but are born live. Although the primitive TE cells

surrounding the early marsupial embryo produce a single cell-layered transport interface that may represent a primitive placenta, it can only provide for the metabolic demands of embryonic/fetal development for a short while, accounting for the short internal gestation period of marsupials (Renfree, 2010).

Eutherian, or true placental, mammals diverged from marsupials approximately 140 million years ago. The placenta is a tremendously successful evolutionary adaptation that has enabled the creation of around 18 taxonomic orders grouped into four superorders. These groups gave rise to many lineages, each of which evolved independently in different geographic locations and in response to differing environmental challenges. There is thus a vast variety of placental forms, and identifying what the original placenta looked like is therefore quite difficult, but it may have been hemochorial, discoid, and labyrinthine (Wildman et al., 2006), similar to that observed in rodents. The placenta is likely the most mutable of all organs, with at least 20 identifiable types, depending on the manner of classification (Benirschke and Kaufmann, 1995). Perhaps not surprisingly, divergence among placental types may have developed a large number of species-specific pathologies, leading to potential limitations in cross-species investigations (Guttmacher et al., 2014).

Internal development supported by a placenta provides many benefits including protection from environmental fluctuations in temperature, oxygen, and osmolarity, as well as protection from predation (Shine, 1989; Clutton-Brock, 1991; Wourms and Lombardi, 1992). It also allows females to produce larger offspring with a higher rate of survival due to enhanced feeding, digestion, movement, or behavior (Amoroso, 1968; Baylis, 1981; Wourms, 1994). However, this also comes with high energetic costs to the mother (Boehlert et al., 1991; Qualls and Shine, 1995). In addition to limiting maternal mobility, increasing time between births, and decreasing frequency of reproduction (Thibault and Schultz, 1978; Goodwin et al., 2002), internal, placenta-supported reproduction demands adaptation of multiple maternal organ systems during pregnancy. The complexity of this benefit–risk ratio may also add to the many placental variations that exist.

Development of the Placenta

Trophoblast Lineage Allocation

The placenta is the first organ to form in mammals. This is because it is required for establishment of a functional maternal–fetal vascular interface capable of supplying the bioenergetic needs of the developing conceptus (Maltepe et al., 2010). The fertilized embryo undergoes a series of cell divisions before implantation, to produce up to eight seemingly identical cells called blastomeres. Three further sets of divisions generate the blastocyst, consisting of two distinct cell populations. Surrounding the blastocyst is the TE, which gives rise to the placenta. The inner cell mass (ICM), located inside the blastocyst, gives rise to the embryo and visceral endoderm (Nishioka et al., 2009; Sasaki, 2010). In mice, each blastomere is able to generate either ICM or TE derivatives and is thus totipotent. This also occurs in humans (Zdravkovic et al., 2015). Once TE or ICM commitment occurs, however, it is considered largely irreversible. Importantly, however, individual blastomeres are found to harbor intrinsic biases regarding which lineage they adopt as early as the four-cell stage (Piotrowska-Nitsche et al., 2005; Tabansky et al., 2013) and which appear to depend on positional cues (Nishioka et al., 2009).

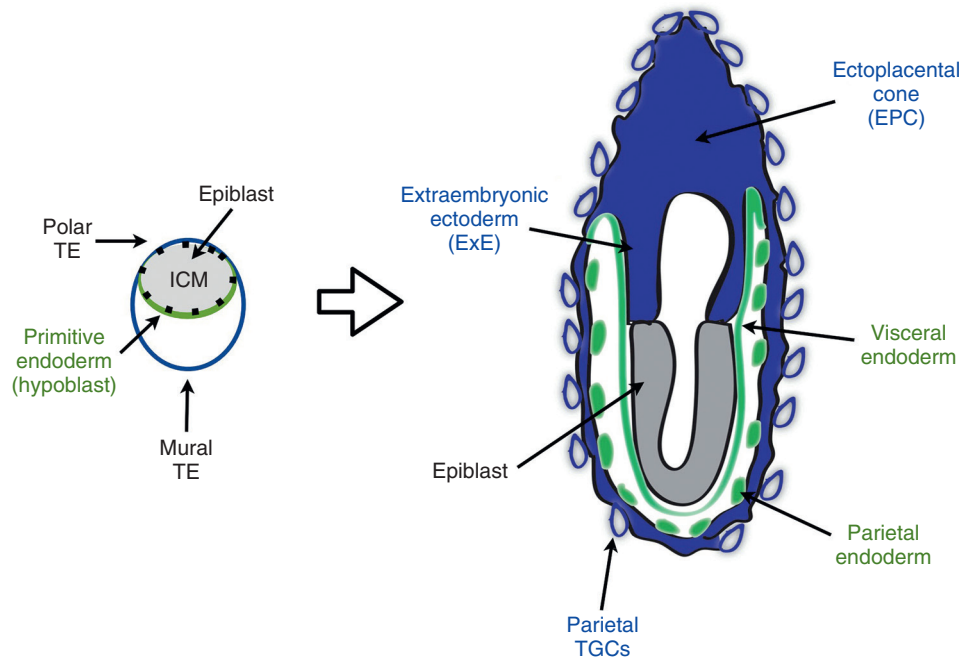
Multiple factors govern lineage allocation. One major determinant includes differences in polarity and adhesion between inner and outer cells of the blastocyst that are associated with differential

activation of the Hippo signaling cascade (Nishioka et al., 2009; Hirate et al., 2013). Hippo helps restrict expression of key lineage regulatory genes such as *Cdx2* that are stochastically expressed as early as the eight-cell stage in mice but restricted thereafter to the TE (Dietrich and Hiiragi, 2008). The Hippo pathway is inactivated in outer TE cells, resulting in nuclear localization of the transcriptional coactivator Yes associated protein (YAP), which interacts with TEA domain transcription factor 4 (TEAD4) to drive Caudal type homeobox 2 (*Cdx2*) gene expression (Nishioka et al., 2008). Notch signaling also acts in parallel with Hippo to promote *Cdx2* expression in this process (Rayon et al., 2014). Positional cues governed by E-cadherin expression help regulate Hippo signaling. Cell–cell contact within inside cells of the ICM activates Hippo and suppresses nuclear YAP activity. Signaling cascades implicated in this include the LATS2 kinase. For example, Hippo can be suppressed by a dominant negative form of the LATS2 kinase or by combined LATS1/2 deficiency. This results in ICM cells exhibiting nuclear YAP localization, *Cdx2* expression, and TE fates (Nishioka et al., 2009), placing LATS1/2 downstream of E-cadherin-dependent signaling. In mice, CDX2 helps repress genes critical for ICM identity, such as *Oct4* and *Nanog*. Its absence in mouse embryos results in the lack of TE differentiation, and all cells of the blastocyst stage embryo express the typically ICM-restricted OCT4 protein (Niwa et al., 2005). This ability is species dependent, however, and is not observed in bovine (Berg et al., 2011) or human blastocysts (Niakan and Eggan, 2013). Amazingly, in mice its expression is sufficient to convert embryonic stem cells (ESCs) into trophoblast stem cells (TSCs) that can contribute to all lineages found within the placenta (Tanaka et al., 1998; Niwa et al., 2005). *Cdx2* expression is further maintained in mice via a positive feedback loop driven by the combinatorial activities of the transcription factors Eomesodermin (EOMES), ETS-related transcription factor 5 (ELF5), ETS proto-oncogene 2 (ETS2), and transcription factor AP-2, gamma (TCFAP2c) that help maintain the TE lineage (Yamamoto et al., 1998; Russ et al., 2000; Auman et al., 2002; Werling and Schorle, 2002; Donnison et al., 2005; Georgiades and Rossant, 2006; Ng et al., 2008; Weber et al., 2010). Interestingly, ELF5, CDX2, and EOMES can collaborate to regulate hundreds of TSC genes by binding to enhancer elements that harbor endogenous retrovirus-derived sequences, indicating that these serve as trophoblast-specific enhancer elements in mice (Chuong et al., 2013). This opens the exciting possibility that differences in the incorporation of these foreign viral elements across different placental mammals may have contributed to the diversity of placental structures seen across the animal kingdom.

Uterine evolution paralleled placental evolution in eutherian mammals. This was recently found to involve large-scale and rapid changes in endometrial gene regulatory networks mediated by ancient transposable elements. These modulate responses to pregnancy hormones as well as other pathways to ensure pregnancy success (Lynch et al., 2011; Lynch et al., 2015). Thus a set of genetic tricks coupled with host–virus interactions enabled the rapid evolution of the placenta–uterus axis in mammals and helped contribute to the diversity of mammalian life forms and modes of procreation observed today.

Trophoblast Differentiation

The placenta is comprised of multiple different cell types that engage in highly varied functions, ranging from attachment, invasion, and vascular remodeling to cell fusion, hormone production, and nutrient transport (Maltepe et al., 2010). Thus trophoblast-specific



• **Fig. 5.1 Embryonic Development in the Mouse Conceptus.** At the blastocyst stage, the conceptus is comprised of an inner cell mass (ICM) that gives rise to the embryo proper (*epiblast*), overlaid by a layer of primitive endoderm (*hypoblast*) that gives rise to the visceral and parietal endoderm. The outer cells comprise the trophoctoderm (TE) that gives rise to the placenta. TE cells near the ICM are referred to as polar TE, while TE cells not in contact with the ICM are referred to as mural TE. The mural TE gives rise directly to parietal trophoblast giant cells in the initial wave of trophoblast differentiation. These cells aid the initial attachment and invasion process. The polar TE differentiates into the ectoplacental cone and extraembryonic ectoderm. The ectoplacental cone resides closest to the implantation site and also gives rise to lineages that further help incorporate the conceptus into the receptive uterus. The extraembryonic ectoderm resides closest to the developing epiblast and gives rise to lineages comprising the placental transport interface, such as the syncytiotrophoblast. TGCs, Trophoblast giant cells.

progenitors need to enact a complex set of lineage restriction decisions to help form a functioning placenta.

The isolation of rodent TSCs, the extraembryonic equivalent of ESCs, has dramatically increased our understanding of cell fate decisions in the placenta (Tanaka et al., 1998). Combined with genetic approaches in mice and analyses of resultant placental phenotypes in vivo, derivation of “knock-out” TSC lines has enabled dissection of lineage commitment decisions within the placenta with great precision. For the human placenta, in vitro experiments with primary cytotrophoblasts (CTBs) as well as ex vivo culture of placental explants have yielded a great deal of information regarding placental development. Human ESCs can also be induced to differentiate into the trophoblast lineage by activating bone morphogenetic protein 4 (BMP4)-dependent signaling pathways (Li and Parast, 2014). Additionally, the recent derivation of single blastomere-derived cell lines from human embryos that can differentiate along the trophoblast lineage is aiding our understanding of human placental development (Zdravkovic et al., 2015). These have been combined with pathologic analyses of placentas following delivery or pregnancy terminations. While primary human trophoblast progenitor cell lines have recently been derived (Genbacev et al., 2011), the exact equivalents of TSCs have not yet been isolated in humans.

TSCs can be derived from mouse blastocysts on fibroblast feeder cells with the addition of fibroblast growth factor 4 (FGF4). Feeder cells produce growth factors such as the TGF- β family member Nodal (Erlebacher et al., 2004; Guzman-Ayala et al., 2004), along

with extracellular matrix-dependent cues (Choi et al., 2013) that maintain TSC proliferation and help prevent their differentiation. In utero, this environment is only maintained for approximately 3 days following implantation, as TSCs cannot be derived from mouse embryos past embryonic day 6.5 (Uy et al., 2002). Removal of growth factor, feeders, or feeder-conditioned medium triggers loss of TSC proliferation as well as markers associated with “stemness” (Roberts and Fisher, 2011). Depending on environmental or culture conditions, these cells then differentiate into the various cells that comprise the placenta. In the mouse, the extraembryonic ectoderm differentiates into cells that comprise the chorion and labyrinth, which perform the transport functions of the placenta, whereas the ectoplacental cone, located nearer to the uterine implantation site, differentiates into the spongiotrophoblast layer as well as glycogen trophoblasts and trophoblast giant cells (TGCs) (Fig. 5.1).

To function as a transport organ, the placenta must establish an extensive vascular interface between the maternal and fetal circulatory systems. Humans and rodents have a hemochorial placenta, which means that the maternal vascular space comes in direct contact with differentiated trophoblasts, not endothelial cells (Benirschke and Kaufmann, 1995). In humans, trophoblasts lining maternal arteriolar spaces are relatively well characterized (Red-Horse et al., 2004), but very little is known about the cells associated with the draining vascular bed, for example. In mice, there appear to be at least five distinct populations of TGCs that lie at various positions within these maternal vascular spaces and are defined by their

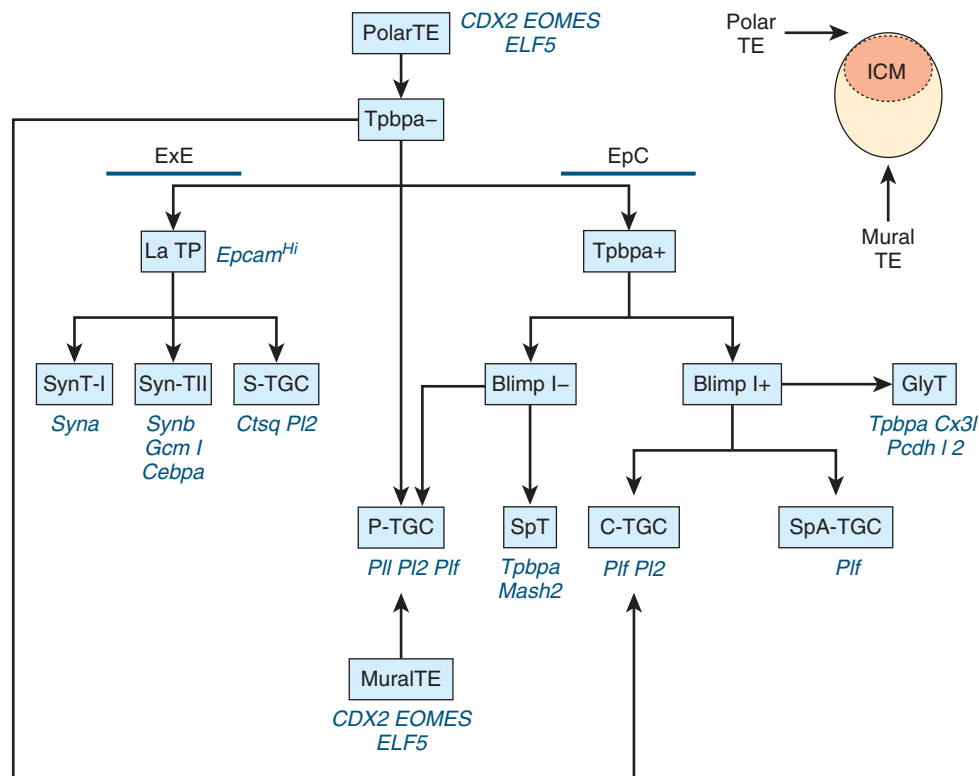
location and lineage-specific gene expression (Simmons et al., 2007). These arise from various sources (see Fig. 5.1). Their “giant” size is, in part, a reflection of their DNA content, which continuously replicates without engaging in cell division via a process called endoreplication (Edgar et al., 2014). Endocycles are thought to be used by cell types that need to be very large or that are highly metabolically active. Consistent with this, TGCs in the mouse placenta are responsible for the bulk of placental hormone production (Soares et al., 2007). In humans, placental hormones are produced by the syncytiotrophoblast (SynT) layer, potentially accounting for the reduced ploidy of invasive trophoblast subtypes in this species compared with rodents, although they still appear to exhibit a significant amount of aneuploidy (Weier et al., 2005).

Trophoblast Invasion

Primary TGCs differentiate from the mural TE, and this represents the first terminally differentiated cell type in mice to aid in implantation. In humans, the initial wave of invasion following implantation is thought to occur via formation of a primitive syncytium, through

which invasive CTBs push following approximately day 13 or 14 of gestation (Cantor and Ginsberg, 2012). This early wave of syncytialization does not occur in rodents. In mice, the remaining secondary TGCs differentiate either from trophoblast specific protein alpha (Tpbpa)/4311+ outer ectoplacental cone cells or from Tpbpa/4311– chorionic progenitors (Simmons et al., 2007) (Fig. 5.2). Secondary TGCs come in various forms that have differing locations as well as characteristics. As the name implies, spiral artery (SpA) TGCs invade the spiral arteries and displace the smooth muscle and endothelial cells to remodel them, canal TGCs line the large vascular spaces delivering maternal blood to the base of the labyrinth, sinusoidal TGCs sit within the small vascular spaces of the labyrinth, and parietal TGCs surround large pools of deoxygenated blood that ultimately drain into the maternal uterine veins.

In addition to their location, these cells can be identified based on lineage-specific gene expression (see Fig. 5.2), although very few unique identifiers are known at this time, and all can be derived in vitro following TSC differentiation (Simmons et al., 2007). A growing list of transcription factors and signaling molecules are

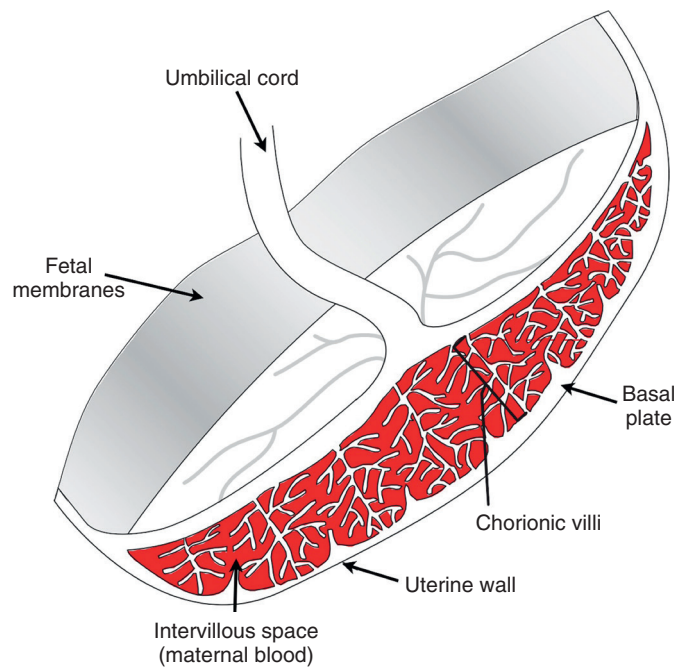


• **Fig. 5.2** Trophoblast Lineage Determination in the Mouse Placenta. Cell type-specific genes are indicated in blue. Mural trophoblast (TE) cells give rise directly to parietal trophoblast giant cells (TGCs). Parietal TE cells, from which trophoblast stem cells (TSCs) are derived, are defined by their expression of key transcription factors including CDX2, EOMES, and ELF5 and can be maintained in vitro by exogenous fibroblast growth factor 4 as well as Nodal signaling. These give rise to all cell types within the mature placenta. Polar TE cells can also give rise directly to parietal TGCs via passage through a trophoblast specific protein alpha (Tpbpa)/4311 negative (–) state. Alternatively, Tpbpa– progenitors can give rise to labyrinth trophoblast progenitors (La TP) characterized by high EpcAM expression that gives rise to lineages that populate the exchange interface. These are the two syncytiotrophoblast (SynT) lineages—SynT-I and SynT-II, as well as the sinusoidal TGCs (S-TGC). Tpbpa+ progenitors, on the other hand, further differentiate through either a Blimp1+ or – state into spongiotrophoblasts (SpT), glycogen trophoblasts (GlyT), or multiple additional types of TGCs, including spiral artery-associated TGCs (SpA-TGCs) and canal TGCs (C-TGCs). Each terminally differentiated cell type is associated with a unique combination of lineage-specific genes that enable their identification in vitro and in vivo (indicated in blue). Epc, Ecto-placental cone; ExE, extraembryonic ectoderm; ICM, inner cell mass; P-TGC, parietal TGC.

known to be critical for these differentiation events and have been reviewed extensively (Hu and Cross, 2010; Maltepe et al., 2010; Pfeffer and Pearton, 2012; Soares et al., 2012; Knofler and Pollheimer, 2013; Knott and Paul, 2014; Latos and Hemberger, 2014; Soncin et al., 2015). An organizational chart depicting the cell fate regulatory hierarchy within the placenta can be made based on our current understanding but still remains poorly characterized when compared with other systems such as hematopoiesis. CDX2-, EOMES-, and ELF5-positive TSCs sit at the apex of this hierarchy (see Fig. 5.2).

Trophoblasts that invade and line blood vessels appear to do so via two different mechanisms: (1) vascular invasion with endothelial mimicry and (2) vasculogenic mimicry (Rai and Cross, 2014). In the former, trophoblasts invade into and displace maternal endothelial cells from within maternal arterioles and include SpA-TGCs in mice or endovascular trophoblasts (EVTs, also known as extravillous trophoblasts) in humans. During vasculogenic mimicry, however, trophoblasts undergo morphogenesis to create vascular tubes de novo. Sinusoidal TGCs perform this function in mice. Whether this also occurs in human placentation is not clear. Transplanting trophoblasts subcutaneously in mice or culturing them as 3-dimensional trophospheres in vitro allows one to visualize them from de novo vascular spaces, where they generate tumors harboring large blood sinuses surrounded by trophoblasts, as opposed to host endothelium (Kibschull and Winterhager, 2006; Rai and Cross, 2014). Many pathways known to regulate endothelial development also drive trophoblast differentiation and formation of the maternal–fetal vascular interface (reviewed in Rai and Cross, 2014). For example, TGCs produce both vascular endothelial growth factor (VEGF) and placenta growth factor (PlGF) and express VEGF receptors -1 and -2, suggesting both autocrine and paracrine roles (Achen et al., 1997; Abbott and Buckalew, 2000; Hirashima et al., 2003). Additionally, Notch signaling, known to be critically important for vascular endothelium, is also important for differentiation of SpA-TGCs and Canal (C)-TGCs in the mouse placenta (Hunkapiller et al., 2011; Gasperowicz et al., 2013). These same factors are also expressed in homologous cells within the human placenta (Zhou et al., 2003a, 2003b; Hunkapiller et al., 2011). Furthermore, the endothelium and trophoblast are primary regulators of hemostasis in the adult and fetal circulation. Trophoblasts can regulate the coagulation cascade like endothelial cells and produce such molecules as thrombomodulin, tissue factor, tissue factor pathway inhibitor, annexin V, and endothelial protein C receptor (Wang et al., 1999; Lanir et al., 2003; Sood et al., 2006). These factors are critical for preventing thrombotic or hemorrhagic events from occurring in the developing placenta. Consistent with this, tissue factor deficiency in mice results in early embryonic lethality associated with massive hemorrhage at this extremely vascular interface (Erlich et al., 1999). Thus mammalian placentas have solved the problem of hemochorial placentation by having trophoblasts take over functions typically performed by endothelial cells.

Remodeling of uterine vasculature is critical for successful pregnancy in humans and mice (Pijnenborg et al., 2006; Maltepe et al., 2010; Soares et al., 2012; Rai and Cross, 2014). The equivalents of TGCs in humans, invasive EVT, are derived from column CTB progenitors located at the tips of anchoring villi (Fig. 5.3). They migrate through the uterine parenchyma via interstitial invasion, in search of maternal spiral arterioles and veins. This invasion peaks early in pregnancy, around 9 to 12 weeks of gestation (Pijnenborg et al., 1981). EVTs then breach the spiral arterioles, via a process termed endovascular invasion, and replace



• **Fig. 5.3** Human Placenta at Mid Gestation. The human (and mouse) placenta is disc shaped (discoid) and hemochorial; i.e., fetal trophoblasts are in direct contact with maternal blood. In contrast to the labyrinthine placenta in mice, the human placenta is comprised of chorionic villi that project into the intervillous space bathed by maternal blood. Fetal blood vessels coursing through the umbilical cord branch when they reach the placenta and dive into the chorionic villi where gas, nutrient, and waste exchange occurs. Tips of anchoring villi attach to the uterine wall (basal plate) and send out waves of invasive cytotrophoblasts (also known as extravillous trophoblasts) to both anchor the placenta and establish blood flow to it. (Adapted from Hunkapiller NM and Fisher SJ. *Methods Enzymol.* 2008;445:281–302.)

resident endothelial and smooth muscle cells (Red-Horse et al., 2006). This results in these high resistance vessels being remodeled into low resistance/high capacitance conduits necessary for proper fetal perfusion as well as modulation of maternal hemodynamics (Red-Horse et al., 2004). While EVT interactions with veins are largely confined to the inner surface of the uterus, they migrate along much of the intrauterine course of maternal arterioles. Although endovascular invasion begins quite early, and typically begins within the center of the placental bed, uterine arterial blood only begins to flow into the intervillous space by the end of the first trimester. Before this point, EVTs paradoxically plug these vessels, preventing blood flow to the placenta. As a result, all of first trimester placental development occurs in a highly hypoxic environment with the bulk of placental nutrients being provided by endometrial secretions (i.e., histiotrophic nutrition) (Burton, 2009). Only about one-third of the uterine SpAs are actually invaded by 18 weeks' gestational age (Pijnenborg et al., 1983), indicating that the more lateral arteries are only invaded throughout the second and third trimesters in a progressive manner (Brosens et al., 2011; Pijnenborg et al., 2011), because most are completely remodeled when examined following delivery at term.

Following unplugging of these vessels, maternal blood begins to bathe floating chorionic villi that are covered by a layer of multinucleated SynTs. SynTs form as a result of the fusion of lineage-committed progenitors. The need for multinucleated syncytium formation is not clear but may have been driven

evolutionarily by a response to viral infections that may help minimize pathogen transmission to the fetus (Tsurudome and Ito, 2000). A combination of fusogenic protein expression, particularly syncytins (Blond et al., 2000; Mi et al., 2000; Malassine et al., 2007; Chen et al., 2008; Esnault et al., 2008; Simmons et al., 2008; Dupressoir et al., 2009; Dupressoir et al., 2011), and dramatic cytoskeletal rearrangement appears to be essential for this trophoblast fusion. Cytoskeletal rearrangement is a common theme in trophoblast differentiation in general (Parast et al., 2001). For example, calponin 3-mediated actin rearrangement can promote SynT fusion (Shibukawa et al., 2010), while caspases can remodel the fodrin cytoskeleton during this process (Gauster et al., 2010), and stathmin expression, a microtubule regulatory protein, is associated with invasive trophoblast migration (Yoshie et al., 2008). Along these lines, invasive TGCs in mice and EVT_s in humans exhibit robust microtubule and actin cytoskeletons, whereas the cytoskeletal network in multinucleated SynT_s found in both species is severely disrupted (Choi et al., 2003; Zhou et al., 2014). Consistent with this, microtubule or actin disrupting agents direct block TGC formation and direct TSC differentiation along the SynT lineage (Choi et al., 2013). Additionally, caspase 8 activity, frequently implicated in apoptosis, aids this process during human SynT formation (Huppertz et al., 1999). These cytoskeletal changes are frequently accompanied by another apoptosis-associated process—externalization of phosphatidylserine to the outer leaflet of the plasma membrane. Typically acting as an “eat me” signal for the clearance of apoptotic cells, phosphatidylserine externalization is associated with SynT fusion (Lyden et al., 1993; Huppertz and Gauster, 2011). Apoptosis is not completed during SynT formation, however, and the syncytium is maintained in this “preapoptotic” state until being sloughed off into the maternal circulation. Other molecules such as connexins are also known to be critical for SynT formation (Kibschull et al., 2008). CD98, an amino acid transporter, also plays a role (Kudo et al., 2003; Kudo and Boyd, 2004; Dalton et al., 2007), and its actions can be opposed by placental protein 13, a galectin family member (Than et al., 2004). In addition to regulating amino acid transport, CD98 can also interact with cell surface integrins to regulate cell morphology and invasion (Cantor and Ginsberg, 2012), playing a unique dual role in the nutrient transport and fusion capabilities of SynT_s. As a result of the dramatic cytoskeletal changes required for cell fusion, in addition to changes in the composition of the membrane lipid bilayer, the biophysical properties of the SynT_s change to become much more rigid, possibly aiding the infection barrier properties of the placenta (Zeldovich et al., 2013).

Analysis of human EVT invasion *in situ* and *in vitro* established that a unique epithelial-to-endothelial switch is a vital component of this process (reviewed in Red-Horse et al., 2004). Initially, CTB progenitors in floating villi express E-cadherin (epithelial cadherin) and integrin $\alpha\beta 4$. Upon differentiation, these cells repress these molecules and upregulate other adhesion molecules such as vascular endothelial cadherin, $\alpha 5\beta 1$, $\alpha V\beta 3$, PECAM-1, and VCAM-1 as well as the matrix metalloproteinase MMP-9. Other data suggest that CTB differentiation/invasion also entails a switch from a venous to an arterial phenotype in terms of the cells' expression of Eph and ephrin molecules (Red-Horse et al., 2005) and the modulation of Notch family members (Hunkapiller et al., 2011). This change is accompanied by an induction of several growth factors and receptors (e.g., VEGF and angiopoietin family members) that function during conventional vasculogenesis and angiogenesis, as well as placental development in other species (Andraweera et al., 2012; Chen and Zheng, 2014).

Placental Functions

Transport

The transport functions of the placenta are performed by the multinucleated SynT_s that sit at the maternal–fetal interface. Multiple mechanisms allow transport of waste and nutrients across the placenta (Dilworth and Sibley, 2013). The simplest is diffusion. The high surface area of the placental transport interface, along with the hemochorial nature of the rodent and human placentas, enables efficient diffusion across the placenta. The rate of diffusion depends on the molecular properties and concentrations of the solute, however, in addition to the composition of the exchange barrier (Sibley and Boyd, 1988). In the human placenta at term, a single SynT layer separates maternal blood from fetal capillary endothelium, whereas in the mouse, two SynT layers as well as an sinusoidal (S)-TGC layer, surprisingly, separate the vascular spaces (Rossant and Cross, 2001). These layers are progressively thinned out to minimize their barrier properties and increase the surface area for exchange (Simmons et al., 2008). Oxygen is transported across the placenta via passive diffusion, aided by the high affinity of fetal hemoglobin and the concentration differential across the maternal–fetal vascular beds. The orientation of the maternal and fetal vascular blood spaces produces a countercurrent exchange mechanism in mice. This maximizes transport efficiency in rodents, whereas the human placenta has a less efficient multivillous arrangement, which necessitate a larger placental size relative to the mouse (Benirschke and Kaufmann, 1995).

Hydrophilic molecules do not readily cross plasma membranes. Transporter protein-mediated mechanisms are typically required for transporting hydrophilic molecules. Classic transporter proteins include facilitated diffusion transporters, i.e., the glucose transporter (GLUT) family (Jansson et al., 1993) as well as active transporters, i.e., transporters associated with calcium transport (Belkacemi et al., 2005) and the amino acid transporters (Desforges and Sibley, 2010). Transport can occur down a concentration gradient, as is the case with GLUT1-mediated glucose transport (Desforges and Sibley, 2010), or against a concentration gradient, as is the case with calcium (Dilworth et al., 2010) and amino acid transport (Battaglia, 2007). Interestingly, nearly all amino acids in the fetal circulation are found at higher levels than in the maternal circulation, indicating active uptake and/or synthesis of these nutrients via the placenta or fetus.

The fetal–placental unit is both physically and metabolically interconnected with each other and the maternal circulation. Ultimately, all fetal–placental metabolism is constrained by the nutrients delivered from the maternal circulation. However, the placenta and fetal liver are both capable of producing and metabolizing various nutrients that impact their levels in the fetal–placental circulation largely independent of placental transport mechanisms (Cetin, 2001). This has been well described in ovine species, wherein the fetal liver of sheep *in utero* actively produces large quantities of serine and glutamate that are consumed by its placenta (Cetin et al., 1992; Chung et al., 1998). In the placenta, serine is converted to glycine via a process that contributes to one-carbon metabolism-dependent DNA methylation pathways that play important roles in cell fate regulation and growth mechanisms (Amelio et al., 2014). Additionally, nonglucose carbohydrates such as fructose, mannose, inositol, and sorbitol are also either transported by or synthesized in the placenta (Jauniaux et al., 2005; Battaglia, 2007) and play important roles in regulating fetal growth as well as in redox regulation.

Finally, antibody-mediated immunity is transferred from mother to fetus across the placenta via receptor-mediated mechanisms (Saji et al., 1999; Schneider and Miller, 2010). Immunoglobulin G (IgG) transport across the human placenta, for example, begins at approximately 16 weeks' gestation, and fetal serum IgG levels reach maternal levels by 26 weeks' gestational age. This process is highly efficient, enabling fetal concentrations to exceed maternal values at term.

Metabolism

The placenta is not an inert transport interface. It consumes 40%–60% of the oxygen and glucose delivered to the uterus at term, despite only comprising approximately 10%–20% of the total mass of the uterus at that time (Bell et al., 1986; Carter, 2000). Changes in this metabolism can regulate placental biology. Mitochondrial fusion, a process that enables greater mitochondrial bioenergetic capacity, is critical for invasive TGC formation in mice, triggering placental failure when compromised (Chen et al., 2003; Alavi et al., 2007). Interestingly, mitochondrial fusion can promote cardiomyocyte differentiation as well, highlighting conserved mechanisms between cellular metabolism and cell fate determination (Kasahara et al., 2013). Thus alterations of placental metabolic function can impact oxygen and nutrient delivery to the fetus, both by altering placental metabolic demand intrinsically, as well as by impacting cell fate regulatory pathways. Primary culture of human CTBs indicates that they exhibit high rates of aerobic glycolysis when compared with other terminally differentiated adult cells (Bax and Bloxam, 1997), much like rapidly proliferating cancer cells that rely on high glycolytic flux rates to augment biosynthetic precursor production. Aerobic lactate production, i.e., the Warburg effect, also allows the placenta to produce and transfer large amounts of lactate to the fetus, which can readily oxidize it. Interestingly, the placenta also appears able to metabolize lactate during mid gestation but loses this ability by term (Carter et al., 1993). These studies additionally suggest that glycogen breakdown (glycogenolysis) helps supply the high rates of glucose consumption in proliferating trophoblasts before differentiation into terminally differentiated SynTs. Interestingly, excess glycogen accumulation has been noted within the SynT layer of some human preeclampsia (PE) placentas (Arkwright et al., 1993), consistent with their altered turnover and suggesting a potential link to altered glucose metabolism in the setting of this pregnancy complication. Importantly, epidemiologic studies confirm that derangements in glucose and fatty acid metabolism may drive pregnancy complications. For example, maternal gestational diabetes mellitus (GDM) and obesity are independently, and additively, associated with elevated rates of PE (Catalano et al., 2012) as well as spontaneous preterm birth (Shaw et al., 2014). There may be shared mechanisms involving impaired trophoblast invasion contributing to PE and preterm labor (PTL) pathogenesis. For example, up to 30% of patients with spontaneous PTL have placental lesions consistent with impaired SpA remodeling typically observed during PE (Kim et al., 2002; Kim et al., 2003; Romero et al., 2014). Thus improving our understanding of the links between placental metabolism and placental development may shed light on the growing epidemic of preterm birth.

Metabolic stressors such as hypoxia at high altitude, or placental underperfusion associated with intrauterine growth restriction (IUGR), alter placental metabolism in particular ways. During isolated hypoxia induced at high altitude, for example, the human placenta appears to decrease its consumption of oxygen in favor

of glycolysis to maintain its bioenergetic needs (Postigo et al., 2009), which preserves placental growth. While preserving fetal oxygen (O₂) delivery, this comes at the expense of glucose, however (Illsley et al., 2010). Given that fetal hypoglycemia is strongly associated with fetal growth restriction, this limits fetal growth. With maternal undernutrition, however, where uterine O₂ delivery is relatively spared, glucose delivery to the fetus is compromised (Coan et al., 2010; Sandovici et al., 2012) in a manner associated with restricted placental growth. Given that the placenta is a complex organ with multiple different cell types (Simmons et al., 2007, 2008), it is likely that changes in placental cellular composition due to alterations in cell fate regulatory pathways play important roles in the reallocation of placental metabolic flux patterns. Consistent with this, maternal calorie restriction leads to a loss of glycogen trophoblasts in the mouse (Coan et al., 2010). Importantly, isolated hypoxia results in increased Hypoxia-inducible Factor-1 (HIF-1) levels and target gene expression in the human placenta (Nevo et al., 2006; Ietta et al., 2007; Zamudio et al., 2007), and HIF activity regulates trophoblast cell fate decisions, suggesting a potential contribution to these pathologic changes (Cowden Dahl et al., 2005; Maltepe et al., 2005; Choi et al., 2013; Zhou et al., 2014). HIF activity can mediate metabolic adaptation to hypoxia via numerous ways (Semenza, 2010), including repressing mitochondrial O₂ consumption while increasing glycolysis and modulating glucose transport as well as amino acid metabolism.

Endocrine Function

In addition to performing the essential transport functions of the placenta in humans, SynTs secrete numerous pregnancy-related hormones (Maltepe et al., 2010). Mammalian placenta produces a greater diversity of hormones in greater quantity than any other single endocrine tissue. Near term, steroid hormones (primarily estrogens and progestins) are being made at the rate of 0.5 grams per day, and protein hormones (lactogens, growth factors, and other hormones similar to those of the hypothalamic–pituitary–adrenal [HPA] axis) are being made at more than twice this rate. Many of these hormones are species specific, but the categories of hormones (i.e., steroids, pituitary-like, hypothalamic-like etc.) and their endocrine, paracrine, and autocrine functions in pregnancy are frequently conserved (Table 5.1).

Steroid Hormones

The placenta is an “incomplete” steroidogenic organ and does not express a complete set of enzymes for de novo production of estrogens and progestins. Steroid hormone synthesis in the placenta is dependent on precursors from mother and fetus, leading to the concept of an integrated *maternal–fetal–placental unit* (Ryan, 1980). Fig. 5.4 diagrams the tissues and enzymes that participate in the biosynthesis of progestins and estrogens (Kallen, 2004). The concentration of steroid hormones in the maternal circulation increases dramatically throughout gestation (Tulchinsky et al., 1972).

Progesterone

Maternal cholesterol, derived from low-density lipoprotein, is transported to the placenta and bound to low-density lipoprotein receptors on SynTs, where it is incorporated by endocytosis and hydrolyzed to free cholesterol in lysosomes (Hussa, 1980). There is no significant 3-hydroxy-3-methylglutaryl coenzyme A activity in human placenta and thus maternal cholesterol must be used

TABLE 5.1 Placental Classification (Incorporating the 2014 Amsterdam Placental Workshop Group Criteria)

1. Placental Vascular Processes
 - a. Maternal stromal-vascular lesions
 - Developmental
 - Superficial implantation/decidual arteriopathy
 - Increased immature extravillous trophoblast
 - Malperfusion
 - Global/partial
 - Early: distal villous hypoplasia
 - Late: accelerated villous maturation
 - Segmental/complete
 - Villous infarct(s)
 - Loss of integrity
 - Abruptio placenta (atrial)
 - Marginal abruption (venous)
 - Acute
 - Chronic
 - b. Fetal stromal-vascular lesion
 - Developmental
 - Villous capillary lesions
 - Delayed villous maturation (maturation defect)
 - Dysmorphic villi
 - Malperfusion
 - Global/partial
 - Obstructive lesions of umbilical cord
 - Recent intramural fibrin in large fetoplacental vessels
 - Small foci of avascular or karyorhectic villi
 - Segmental/complete
 - Chorionic plate or stem villous thrombi
 - Large foci of avascular or karyorhectic villi
 - Loss of integrity
 - Large vessel rupture (fetal hemorrhage)
 - Small vessel rupture (fetomaternal hemorrhage)
 - Villous edema
2. Placental Inflammatory Immune Processes
 - a. Infectious inflammatory lesions
 - Acute
 - Maternal inflammatory response: chorioamnionitis, subchorionitis
 - Fetal inflammatory response: chorionic/umbilical vasculitis
 - Chronic
 - Villitis (CMV, others)
 - Intervillositis (malaria, others)
 - b. Immune/idiopathic inflammatory lesions
 - Villitis of unknown etiology and related/associated lesions
 - Chronic villitis
 - Chronic chorioamnionitis
 - Lymphoplasmacytic deciduitis
 - Eosinophil T-cell fetal vasculitis
 - Chronic histiocytic intervillitis
3. Other Placental Processes
 - Massive perivillous fibrin(oid) deposition (maternal floor infarction)
 - Abnormal placental shape or umbilical insertion site
 - Morbidly adherent placentas (accreta)
 - Meconium-associated changes
 - Increased circulating nucleated red blood cells

CMV, Cytomegalovirus.

From Redline, R.W. Classification of placental lesions. *Am J Obstet Gynecol*. 2015 Oct;213(4 Suppl):S21-8.

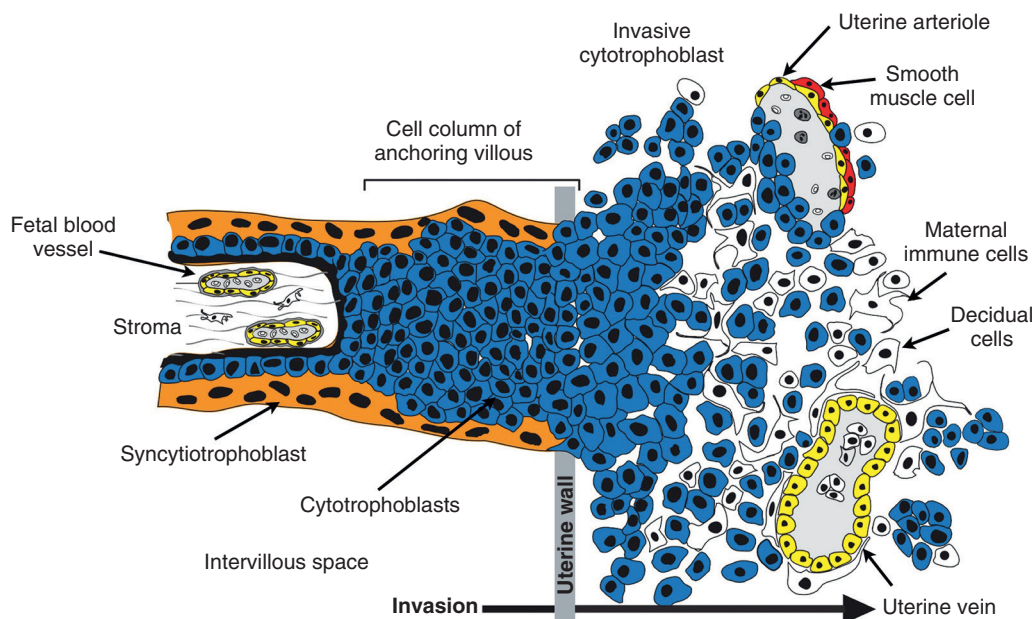
for production of pregnenolone, the first step in steroid synthesis. Cholesterol is converted to pregnenolone in the mitochondria by cytochrome P450 cholesterol side-chain cleavage enzyme. After transfer to the cytosol, progesterone is produced from pregnenolone by type-1 3β -hydroxysteroid dehydrogenase (Osathanondh and Tulchinsky, 1980; O'Connor et al., 1998). Before the ovarian-placental shift, the corpus luteum of pregnancy is the primary source of progesterone, but by 35 to 47 days postovulation the placenta produces enough progesterone to maintain pregnancy (Csapo et al., 1973). The majority (>90%) of progesterone goes to the mother and the rest to the fetus. A limited amount of pregnenolone is also released into the circulation. The fetus has the enzyme activity needed for pregnenolone synthesis but has minimal ability to produce progesterone. High levels of circulating fetal progesterone are of placental origin so that circulating progesterone levels thus reflect placental function, not fetal wellbeing.

Progesterone can be metabolized to 17-hydroxyprogesterone (17-OHP), but relative efficiency of the enzymes favors progesterone production. 17-OHP levels do rise in the third trimester as progesterone levels peak. Additional progesterone metabolites, particularly 5-dihydroprogesterone (5-DHP) and its metabolite allopregnanolone, are also produced in the SynT at increased levels during gestation (Dombroski et al., 1997). These steroids have been hypothesized to play an endocrine role in fetal brain development and provide neuroprotection in the face of hypoxia (Pasca and Penn, 2010; Hirst et al., 2014).

Progesterone (da Fonseca et al., 2003) or a synthetic form of 17-OHP (Meis et al., 2003) is used therapeutically in gestation, as an adjunct for pregnancy maintenance after in vitro fertilization or in the second half of gestation for prevention of preterm delivery in women with a prior history of preterm birth (Di Renzo et al., 2012). Progesterone is required for the maintenance of pregnancy in part by means of its suppressant effect on uterine contractions (Csapo et al., 1973; Kallen, 2004; Ragusa et al., 2004). Progesterone inhibits genes that promote contractility (Mesiano et al., 2002) and has immunosuppressive activity that may promote uterine quiescence (Ragusa et al., 2004; Hardy et al., 2006). Progesterone also counteracts uterine estrogen effects. Unlike the drop in progesterone levels prior to labor seen in most mammals, there is no progesterone withdrawal per se that occurs before labor in women; however, modulation of progesterone receptor expression in combination with a shift in the progesterone to estrogen balance is presumed to play the same biological role. The relationship of therapeutic response to normal physiologic mechanisms at work in the maternal-fetal-placental unit is not yet understood.

Estrogens

Unlike the requirement for maternal precursors for progesterone production, estrogen production relies on fetal precursors. In pregnancy, estrogens are synthesized from C19 steroids (Siiteri and MacDonald, 1966), primarily from dehydroepiandrosterone sulfate (DHEA-S) made in the fetal adrenals. The fetal adrenals rapidly inactivate steroids through sulfatization. Pregnenolone is sulfated and converted to DHEA-S (Benirschke et al., 1956), which then may be hydroxylated in the fetal liver. These biologically inactive androgens are then transferred back to the placenta. Placental sulfatases rapidly cleave the sulfate, and placental 3β -hydroxysteroid dehydrogenase converts DHEA or hydroxylated DHEA to androstenedione or hydroxylated androstenediones, respectively. These androgens are then aromatized to estrone (E1),



• **Fig. 5.4** Basal Plate of the Human Placenta at Mid Gestation. A multinucleated layer of syncytiotrophoblasts (SynTs) covers the finger-like projections of chorionic villi. SynTs perform all of the transport functions of the placenta. A single cell layer of cytotrophoblasts (CTBs) sits just below the SynT layer and is referred to as villous CTB. These cells fuse with each other as they differentiate into SynTs. At the tips of anchoring villi sits a column of CTBs that are proliferative and that put out waves of invasive CTBs (extravillous trophoblasts) that help anchor the placenta to the uterus. Importantly, these also invade maternal spiral arterioles, where they replace the endothelial lining, induce the apoptotic death of surrounding smooth muscle cells, and transdifferentiate into an endothelialized cell type. This invasion typically courses through the extent of the uterine myometrium. CTBs can also invade veins but do not appear to remodel them. Additionally, maternal uterine immune cells, such as natural killer cells, appear to play an important role in enabling CTBs to invade the uterus and its vasculature. Finally, establishment of a receptive deciduum is also of critical importance for proper placental development and pregnancy success. (Adapted from Genbacev O, et al. *Dev Biol.* 2001 May 15;233[2]:526-36.)

16 α -OH estrone, or 15 α -OH estrone and then converted to estradiol (E2), estriol (E3), or estetrol (E4) respectively by placental 17 β -hydroxylation (Jameson et al., 1986; Morrish et al., 1987; Shi and Zhuang, 1993). E3 is the major estrogen of pregnancy with the majority secreted into the maternal compartment; E1 is the only estrogen preferentially secreted into the fetal compartment. Although maternal DHEA-S serves as 40% of the precursor for E2 synthesis, E3 and E4 are formed predominantly from fetal precursors because the maternal liver has limited 5 α -hydroxylation or 16 α -hydroxylation capabilities (Madden et al., 1978). E3 and E4 are thus indicators of fetal function (Tulchinsky et al., 1972), although neither is a clinically useful marker because of rapid shifts in circulating levels. The primary function of high E3 levels remains unclear, but it does increase uteroplacental blood flow (Resnik et al., 1974).

Estrogens influence uterine growth, blood flow, contractility, metabolism, and breast development (Branchaud et al., 1983). However, high estrogen levels are not apparently needed for pregnancy. Parturition can proceed in the absence of fetal and placental sulfatase (Bradshaw and Carr, 1986) or aromatase (Harada, 1993), although in the latter case both fetus and mother are virilized. In such pregnancies, there is still circulating estradiol. There are no reports of pregnancy without detectable estrogen levels, suggesting that a basal level of estrogen is likely required. Before parturition, an increase in the estrogen to progesterone ratio occurs within the intrauterine tissues and may increase prostaglandin (PG) and oxytocin (OT) activity. Steroid hormone production is

altered by trophic hormones and other factors, including hypothalamic-like releasing or inhibiting hormones. In turn, estrogens affect other endocrine systems (i.e., renin-angiotensin system) (Carr and Gant, 1983) and support organ maturation such as surfactant production in the lung (Parker et al., 1987).

Glucocorticoids

In addition to the sex steroids, circulating levels of glucocorticoids and mineralocorticoids are increased in pregnancy (Dorr et al., 1989). The placenta has the ability to produce cortisol and to convert it to inactive cortisone via 11 β -hydroxysteroid dehydrogenase type 2. This enzyme also converts maternal cortisol to cortisone at the placental interface. The primary role of this system appears to be to protect the fetus from elevated cortisol exposure, which may play a role in long-term reprogramming of the fetal HPA axis (Challis et al., 2001). Dual oxidative and reductive enzymatic activity regulates the balance between cortisol and cortisone (Pepe and Albrecht, 1995). In the placenta, oxidation of cortisol to cortisone predominates, whereas in the decidua, the reverse reaction dominates, potentially providing localized hormone exposure.

Pituitary-Like Hormones

A combination of pituitary-like growth hormones are required to support fetal growth while maintaining maternal metabolic homeostasis.

Human Chorionic Gonadotropin

Human chorionic gonadotropin (hCG) is one of the first hormones of pregnancy, produced by trophoblasts even before placenta formation (Hay and Lopata, 1988) and is unique to human pregnancy (Maston and Ruvo, 2002). After placentation, hCG is synthesized primarily by the SynT (Midgley, 1962) and passes into the maternal circulation via secretion into the intervillous space. hCG is a glycoprotein heterodimer (36 to 40 kDa) composed of α and β subunits encoded by genes on chromosome 6 and 19, respectively (Hussa, 1980; Fournier et al., 2015). The α subunit is homologous to pituitary thyroid-stimulating hormone (TSH), luteinizing hormone (LH), and follicle-stimulating hormone (FSH), while the β subunit is homologous to LH. Intact hCG (i.e., having both α and β subunits) is required for hCG endocrine activities. Since it shares a receptor with LH, the LH chorionic gonadotrophin receptor (LHCGR), hCG mimics the function of LH, but the functions of LH and hCG are quantitatively different due to the longer half-life of hCG and its relative stability compared with the pulsatile release of pituitary LH (Muyan and Boime, 1997).

hCG maintains corpus luteal progesterone production until this function shifts to the maturing placenta. hCG peaks approximately 2 weeks after the shift of progesterone production from ovary to placenta, potentially minimizing the chance of loss of the progestational environment. hCG can be detected in human serum or urine within a week of conception and is the most frequently used biochemical marker for pregnancy. hCG doubling time may be used in early gestation to predict general pregnancy outcome. After hCG can first be detected, it increases with a doubling time averaging 2.11 days. It reaches peak levels of approximately 50 international units (IU)/mL at 9 to 10 weeks from the date of the last menstrual period, declining to 1 IU/mL by mid gestation (Osathanondh and Tulchinsky, 1980). An abnormally slow doubling time of hCG is considered to be a sign of a poor prognosis for pregnancy outcome, while rising hCG without detection of an intrauterine embryo suggests an ectopic pregnancy (Fritz and Guo, 1987).

In addition to corpus luteum maintenance, hCG has multiple additional activities that regulate placental structure and function. hCG acts as an autocrine signal in trophoblasts expressing LHCGR promoting the differentiation of SynTs, thus amplifying its own production since it is made primarily by these cells (Shi and Zhuang, 1993). Phosphorylation of the receptors via this pathway also decreases LHCGR expression in differentiating SynTs, thus completing a feedback loop (Pidoux et al., 2007). hCG may also have roles in endometrial angiogenesis, uterine quiescence, and immunotolerance to the fetus (Osathanondh and Tulchinsky, 1980; Fritz and Guo, 1987; Fournier et al., 2015). In addition, hCG can alter maternal TSH levels, elevating free thyroxine (T₄), although this increase does not appear to cause maternal hyperthyroidism (Challis et al., 2009).

Glycosylation state and subunit availability regulate hCG activity. A hyperglycosylated form (hCG-H) has been detected in early pregnancy, as well as in choriocarcinoma cells. hCG-H appears to enhance trophoblast invasion (Fournier et al., 2015) and thus may be a very early biomarker of placental invasion of the endometrium. A decreased level of hCG glycosylation in very early pregnancy has been correlated with early pregnancy loss (O'Connor et al., 1998; Kovalevskaya et al., 1999; Fournier et al., 2015). Isoform production may also regulate activity. β -Subunit production exceeds α -subunit production in early pregnancy, but this ratio rapidly shifts to α -subunit excess, increasing as gestation progresses; circulating hCG is mostly intact hCG or free α -hCG. It has been proposed

that ratios of hCG isoforms (intact hCG, independent subunits, and nicked breakdown products) present in maternal blood and urine might be useful for detection of pregnancy-related disorders since only intact hCG is fully active (Montagnana et al., 2011).

Local and systemic factors influence hCG production. Locally, its expression is regulated by a releasing factor, gonadotropin-releasing hormone (GnRH) 1 and 2 (Khodr and Siler-Khodr, 1978; Khodr and Siler-Khodr, 1980; Siler-Khodr and Grayson, 2001). Neurotransmitters (Shi and Zhuang, 1993), cyclic adenosine monophosphate (Jameson et al., 1986), epidermal growth factor (EGF) (Morrish et al., 1987), activin (Steele et al., 1993), cytokine (Wegmann and Guilbert, 1992), and PGs (Licht et al., 1993) regulate hCG production, as does hCG itself as noted previously. Each of these factors is produced by the placenta, as well as by other extraembryonic tissues. hCG alters placental steroidogenesis by stimulating both progesterone and estrogen formation. Estrogens inhibit GnRH stimulation of hCG (Branchaud et al., 1983), thereby completing a feedback axis in the placenta.

Human Chorionic Somatomammotropin

Human chorionic somatomammotropin (hCS), originally known as human placental lactogen (Higashi, 1961; Josimovich and Maclaren, 1962), has both growth hormone-like and lactogenic activity. hCS is detectable in extraembryonic tissues within 10 days of conception and in maternal serum by the third to fourth week of gestation. It is a single 191 amino acid nonglycosylated peptide chain with considerable homology to growth hormone (GH) (96%) and PRL (67%); it is transcribed from a gene cluster on chromosome 17 containing two genes for hCS, one hCS pseudogene and two GH genes (Rygaard et al., 1998; Handwerger and Freemark, 2000). SynTs produce hCS at a constant rate during gestation, thus hCS levels reflect total placental mass and gross placental function. By term, hCS is made at 1 gram per day, representing 10% of total placental protein synthesis.

hCS is considered one of the major diabetogenic factors of pregnancy, along with placental steroids, placental GH variant (hGH-V), and maternal cortisol. It is almost exclusively found in maternal rather than fetal circulation. This has led to the hypothesis that the primary role of hCS is to ensure adequate fetal nutrition because, in maternal circulation, it induces metabolic changes such as mobilization of fatty acids, insulin resistance, decreased utilization of glucose, and increased availability of amino acids through decreased maternal use of protein (Grumbach et al., 1968). Circulating maternal glucose and free fatty acids are thus increased. While glucose readily crosses the placenta, fatty acids cross slowly, thus biasing glucose delivery toward the fetus and use of fatty acids for maternal energy, especially during maternal fasting. Within the placenta, hCS may regulate insulin-like growth factor (IGF-1) (Kanda et al., 1998) and alter fetal growth through direct action on placental nutrient transport systems. In addition to its metabolic activity, the lactogenic activity of hCS may prepare the breast for lactation, working synergistically with PRL and steroids (Alsatt et al., 1998). Most recently, a role for hCS as a placental angiogenic factor has been suggested (Corbacho et al., 2002).

Placental Growth Hormone Variant

hGH-V is encoded in the same chromosome 17 gene cluster as hCS and pituitary GH. Two transcripts are generated from the hGH-V gene, a major form and an alternatively spliced version. Secreted hGH-V is translated from the major version and is produced in a highly bioactive 22 kD nonglycosylated form and to a lesser degree in a 25 kD glycosylated form (Alsatt et al., 1998).

Early in pregnancy, maternal pituitary GH is produced, but from 15 to 20 weeks' gestation to term hGH-V secretion increases, suppressing maternal GH. hGH-V peaks about a month before term delivery and disappears from maternal circulation immediately after delivery (Newbern and Freemark, 2011). hGH-V is not detected in the fetal circulation. Much like hCS, hGH-V modifies maternal metabolism to meet fetal needs. hGH-V primarily appears to control maternal IGF-1 production (Newbern and Freemark, 2011). In mice overexpressing hGH-V (not normally found in rodents), body weight was increased, IGF-1 levels were elevated, and insulin resistance developed, suggesting that hGH-V strongly contributes to the insulin resistance of pregnancy (Barbour et al., 2002) and increases the risk of gestational diabetes and other pregnancy-related pathologies. This risk is counterbalanced by placental lactogens, hCS and PRL, which induce increased insulin secretion by pancreatic β -cell expansion. hGH-V secretion is tonic, in contrast to pulsatile pituitary GH, and is not regulated by hypothalamic releasing factors (Newbern and Freemark, 2011). Secretion is inhibited by elevated glucose and mildly increased by hypoglycemia, creating a feedback loop that may ensure constant delivery of nutrients to the developing fetus.

Insulin-Like Growth Factors

IGF1 and IGF2, are the primary somatotrophs in gestation. IGFs are highly homologous single chain polypeptides with similarities to pro-insulin made in human placental tissues (Han and Carter, 2000). The majority of the components of the insulin/IGF system are found in the placenta (IGF1, IGF2, and the IGF-binding proteins [IGFBP] 1–6) (Forbes and Westwood, 2008) except insulin itself, although maternal insulin has profound indirect effects on fetal growth and wellbeing. hGH-V levels regulate placental IGF levels (Kanda et al., 1998), and IGFBPs are carrier proteins expressed in the human placenta that prevent IGF from degradation while blocking bioactivity (Hill et al., 1993; Han and Carter, 2000; Forbes et al., 2008). IGFBP1 is also produced by the decidua in large amounts. IGFBPs are themselves regulated by protease activity and through posttranslational modifications, adding a further layer of regulatory complexity.

IGF1 is expressed predominantly in SynT throughout gestation, with some CTB expression, while IGF2 is expressed only in CTB with a declining level across gestation (Hill et al., 1993; Han and Carter, 2000; Forbes and Westwood, 2008; Newbern and Freemark, 2011). At physiologic concentrations, both IGF1 and IGF2 bind to the IGF1 receptor (IGF1R). The localization of IGF1R shifts during gestation; initially it is predominantly expressed on SynTs (closer to the maternal circulation), and by term it is mainly expressed on the fetal CTB side, reflecting the shifting activity from maternal to fetal growth control (Forbes and Westwood, 2008). The IGF2 receptor (IGF2R; also known as the cation-independent mannose-6-phosphate receptor) controls extracellular IGF2 concentrations by mediating the endocytosis and degradation of IGF2, rather than by direct signaling via the receptor (Gicquel and Le Bouc, 2006). An additional receptor, possibly a variant of the insulin receptor, may mediate some of the fetal growth effects of IGF2 (Baker et al., 1993).

Information on the role of IGFs in fetal growth comes from genetic manipulation in mouse models as well as examination of human tissues, especially from fetal growth restricted pregnancies (Gicquel and Le Bouc, 2006). Disruption of mouse Insulin-like growth factor (*Igf1*), *Igf2*, or insulin-like growth factor 1 receptor (*Igf1r*) genes retards fetal growth (Baker et al., 1993), while disruption of *Igf2r* or overexpression of IGF2 enhances fetal growth

(Eggenschwiler et al., 1997). In humans, mutations in the *IGF1* or *IGF1R* genes are extremely rare, and no *IGF2* gene deletions have been reported (Gicquel and Le Bouc, 2006). However, *IGF2* is an imprinted gene normally expressed exclusively from the paternal allele in placenta and fetal tissues. Changes in *IGF2* expression because of abnormal imprinting have been linked to both overgrowth (Beckwith–Wiedemann syndrome) and growth retardation (Russel–Silver syndrome) (Gicquel and Le Bouc, 2006). Whether placentally derived IGFs, versus fetal IGFs, directly contribute to these fetal growth changes is uncertain since these factors also have paracrine effects in the placenta that determine nutrient transport and placental growth.

IGF1 can promote SynT differentiation, while IGF2 does not appear to have this function despite its very early placental expression. In vitro experiments suggest that placental mass is regulated directly by placental IGFs (Forbes et al., 2008); in vivo, loss of IGF2 reduces the placental surface area available for gas and nutrient exchange more than IGF1 loss. Both IGFs increase nutrient transport, especially of amino acids, which may be reflected in elevated fetal amino acids associated with gestational diabetes (Cetin et al., 2005; Forbes et al., 2008). IGFs may alter fetal growth through additional mechanisms since they potentiate EGF activity (Bhaumick et al., 1992), increase prolactin and progesterone production (Nestler, 1987; Kubota et al., 1991), and inhibit placental thromboxane production (Siler-Khodr et al., 1995).

Other Secreted Growth Factors

Platelet-derived growth factor A, transforming growth factor (TGF)- α , and TGF- β (Rappolee et al., 1988) expression in blastocysts appear to be involved in implantation. Other growth factors, including EGF, basic FGF, nerve growth factor, granulocyte colony-stimulating factor, and hepatocyte growth factor as well as growth factor receptors are expressed by the placenta and membranes at later gestation stages (Chegini and Rao, 1985; Stewart, 1996; Uehara and Kitamura, 1996; Morrish et al., 1998). The actions of many of these growth factors may be nonclassical autocrine actions. For example, EGF is made in SynTs, and EGF receptors on SynTs correlate with trophoblast differentiation rather than proliferation (Maruo and Mochizuki, 1987; Mitchell, 1987; Marziani et al., 2005). Additional growth factor actions on placental development are under intensive investigation.

Inhibin and Activin

Inhibin and activin, an antagonist and agonist of pituitary FSH, respectively, are expressed by CTBs and fetal membranes (Petraglia et al., 1987b; Petraglia, 1997) while activin receptors are expressed in SynT (Florio et al., 2004a). Inhibin inhibits hCG and reduces progesterone production (Petraglia et al., 1987b), while activin has the opposite effect (Petraglia et al., 1989). Inhibin elevation is associated with fetal trisomy 21, while elevated activin is associated with PE and gestational diabetes (Florio et al., 2004a). Thus during pregnancy these hormones may serve as potential biomarkers of placental pathologies.

Proopiomelanocortin Hormones

Pituitary-like peptides derived from proopiomelanocortin (POMC), including adrenocorticotrophic hormone (ACTH), melanocyte-stimulating hormone, β -endorphins, and β -lipoproteins, as well as full length POMC itself, are found in the human placenta (Krieger, 1982; Raffin-Sanson et al., 1999). The processing of POMC in the placenta is different from in the pituitary: POMC is released largely intact from the placenta, while it is cleaved into

several peptide hormones in the nonpregnant state. While pituitary POMC-derived peptides respond to and regulate physiologic stress, placental POMC is not inhibited by glucocorticoids nor do circulating levels correlate with ACTH or cortisol levels, although they do correlate with corticotrophin-releasing hormone (CRH) levels (Raffin-Sanson et al., 1999). Chorionic CRH is produced by the placenta and stimulates the release of chorionic ACTH (see later) (Reis et al., 1999). The physiologic role of chorionic ACTH has not been defined, but it may affect placental cortisol production or maternal resistance of ACTH suppression by glucocorticoids.

Hypothalamic-Like Hormones

Every known hypothalamic releasing or inhibiting hormone has a placental analogue (Khodr and Siler-Khodr, 1978, 1980; Siler-Khodr, 1993; Siler-Khodr and Grayson, 2001). These hormones act in placental paracrine–autocrine regulatory networks that control release of placental endocrine hormones.

Gonadotropin-Releasing Hormone

In the placenta, chorionic GnRH, which regulates the paracrine axis, is important for early pregnancy maintenance, as well as regulating gonadal steroid production through stimulation of pituitary LH and FSH (Pawson et al., 2003). Two isoforms of GnRH (GnRH1 and GnRH2) are produced (Seeburg and Adelman, 1984; Siler-Khodr and Grayson, 2001; Sasaki and Norwitz, 2011). GnRH1 is encoded on chromosome 8 as a precursor protein that includes a signal sequence, the GnRH decapeptide, a processing sequence, and a GnRH-associated peptide (Cheng and Leung, 2005). GnRH2 is encoded on chromosome 20 and has 70% homology to GnRH1. GnRH1 and GnRH2 signal through the same G protein coupled receptor, GnRHR1, expressed in SynTs, but may activate different intracellular signaling pathways (Haning et al., 1982; Chou et al., 2003; Sasaki and Norwitz, 2011). Blocking GnRHs or GnRHR1 activity can lead to pregnancy failure (Das and Talwar, 1983; Jagannadha Rao et al., 1985; Sridaran, 1986; Kang et al., 1989), possibly through alteration of hCG and placental steroids, whose production and release they modulate (Siler-Khodr et al., 1986b). The release of placental GnRH1 is affected by cyclic adenosine monophosphate, PGs, epinephrine (Petraglia et al., 1987a), and inhibin (Higashi, 1961), while the expression of GnRHR1 is regulated by GnRH1, activin, and inhibin, creating a feedback loop (Sasaki and Norwitz, 2011).

Corticotrophin-Releasing Hormone and Urocortins

Chorionic CRH and CRH receptors are expressed in placenta and fetal membranes (Shibasaki et al., 1982; Frim et al., 1988; Riley and Challis, 1991; Florio et al., 2000). Urocortins, members of the CRH-hormone family, are also produced and bind to CRH receptors as well (Florio et al., 2004b). Early in gestation, CRH family members may promote immune tolerance (Makrigiannakis et al., 2004). As gestation progresses, CRH and urocortin levels rise peaking at term with delivery (Florio et al., 2004b). These hormones stimulate POMC-derived hormones, including ACTH and β -endorphinins, (Margioris et al., 1988; Florio et al., 2004b), as well as PG release, suggesting roles in parturition (Jones and Challis, 1989). CRH can also stimulate fetal adrenal estrogen and glucocorticoid production (Mesiano and Jaffe, 1997), which may contribute to the timing of parturition. Glucocorticoids can increase placental CRH expression (Jones et al., 1989), in contrast to glucocorticoid inhibition of CRH in the hypothalamus, creating a positive feedback loop that amplifies CRH activity (Nicholson and King, 2001). Because of its tight

association with delivery timing, CRH is often viewed as a *placental clock* (McLean and Smith, 2001) and may be biomarker of pregnancy pathology. In pregnancies complicated by hypertension, the maternal circulating levels of CRH are already elevated by 28 weeks of pregnancy whereas local urocortin levels may be decreased (Petraglia et al., 1996; Florio et al., 2004b). CRH has been proposed as a predictor for preterm delivery (Holzman et al., 2001; McLean and Smith, 2001), but significant clinical utility has not yet been demonstrated (McGrath and Smith, 2002; Smith and Nicholson, 2007).

Thyrotropin-Releasing Hormone

Chorionic thyrotropin-releasing hormone (Shambaugh et al., 1979) is made by the placenta and fetal membranes, but a clear role for either the mother or the fetus has not been identified. Pituitary TSH does not cross the placenta, nor does the placenta make thyroid hormone itself, but maternal T4 and triiodothyronine (T3) cross the placenta carried by placentally produced transthyretin (Landers et al., 2009; Li et al., 2012; Forhead and Fowden, 2014). The placental role in thyroid metabolism has been of considerable recent interest since thyroid disease is common in women of childbearing age and impacts pregnancy outcomes (Nathan and Sullivan, 2014). Early maternal hypothyroidism appears to be associated with lower intelligence quotient in offspring, but conflicting reports exist on the impact of maternal hypothyroidism after onset of fetal thyroid function in mid gestation (Chan et al., 2009; Nathan and Sullivan, 2014). Maternal T4 continues to cross from maternal to fetal circulation in the second and third trimesters; even fetuses with complete thyroid dysgenesis have 30%–50% normal T4 levels in cord blood (Vulsma et al., 1989). Placenta regulation of thyroid hormone transport and metabolism may play a critical role in fetal wellbeing, but the regulatory pathways remain to be defined.

Growth Hormone-Releasing Hormone, Somatostatin, and Ghrelin

Additional releasing factors are made in the CTB, including growth hormone-releasing hormone (Berry et al., 1992), somatostatin (Nishihira and Yagihashi, 1978), and ghrelin (Gualillo et al., 2001) and may regulate hGH-V production as well as placental differentiation (Fuglsang et al., 2005).

Leptin

Leptin is normally secreted by adipocytes and decreases food intake through hypothalamic actions, but in pregnancy, the placenta is the primary leptin source (Ashworth et al., 2000; Linnemann et al., 2001). The precise roles of leptin in the placenta, the mother, or the fetus are not known but may differ significantly from the nonpregnant state, as leptin levels in pregnancy do not correlate with body mass nor produce satiety (Henson and Castracane, 2000; Hauguel-de Mouzon et al., 2006). Increased leptin levels are seen in PE and gestational diabetes (Miehle et al., 2012; Tessier et al., 2013).

Oxytocin

OT is another hypothalamic hormone produced in the placenta and membranes (Gimpl and Fahrenholz, 2001). OT is a potent uterotonic hormone, used clinically to induce or speed labor. However, neither circulating maternal OT nor locally produced OT appears to increase markedly before labor; rather uterine response to OT is increased through increases in OT receptor (OTR) expression and function (Fuchs et al., 1995; Mitchell and

Chibbar, 1995). Progesterone suppresses OTR signaling during gestation (Gimpl and Fahrenholz, 2001), but a decline in progesterone activity at term (although not absolute progesterone levels in humans) increases OTR expression making the uterus more responsive to OT.

Additional Placental Secreted Factors

Vasoactive Peptides

The angiotensin–renin system has been described in the placenta and is thought to be a factor in the regulation of vascular tone in the placental bed. Multiple vasoactive peptides—VEGF, endothelin, angiotensin, arginine vasopressin, and atrial natriuretic peptide—and their receptors are placentally expressed (Myatt et al., 1992; Chao et al., 1993; Kingdom et al., 2000; VanWijk et al., 2000; Kaufmann et al., 2004). A balance of these factors is likely required for appropriate fetoplacental perfusion. For example, atrial natriuretic peptide inhibits the vasoconstrictive action of endothelin and angiotensin and induces vasodilatation in the uterus and the placenta.

Endogenous Opioid Peptides

Opioid peptides, enkephalins (Tan and Yu, 1981) and dynorphin (Lemaire et al., 1983), and their receptors are expressed in placenta, with an increase in placental receptors at term.

Cytokines

Cytokines—interferons, tumor necrosis factor- α (TNF- α), leukemia inhibitory factor, and interleukins (Chaouat et al., 2002; Kimber, 2005; Paulesu et al., 2005; Piccinni, 2005; Hauguel-de Mouzon and Guerre-Millo, 2006)—and their receptors are produced by the placenta, as well as by uterine endothelial cells and invading macrophages (Jokhi et al., 1997; Sel'kov et al., 2000; Piccinni, 2005; Varla-Leftherioti, 2005). Successful implantation requires a proinflammatory cytokine environment (Dealtry et al., 2000; Loke and King, 2000), while pregnancy maintenance requires cytokine expression that suppresses the maternal immune response (Keelan et al., 1999; Challis et al., 2009). Before parturition, this balance again shifts back to proinflammatory cytokines (Jokhi et al., 1997; Keelan et al., 1999; Sel'kov et al., 2000; Varla-Leftherioti, 2005). The balance of cytokines and related factors, either proinflammatory or antiinflammatory, may be a key trigger for preterm labor caused by intrauterine infection or other types of inflammation (Sel'kov et al., 2000; Challis et al., 2009). Cytokine expression also regulates trophoblastic and vascular placental function (Saito, 2001). Cytokines affect these activities by regulation of other cytokines, growth factors, hormones, and prostanoid production (Lundin-Schiller and Mitchell, 1991; Laham et al., 1997; Mohan et al., 2001).

Eicosanoids

Eicosanoids, such as thromboxanes (TXAs), PGs, and leukotrienes, are inflammatory mediators expressed in placenta that are derived from arachidonic acid (Harper et al., 1983; Majed and Khalil, 2012). Human term placentas convert arachidonic acid primarily to TXAs and the PGs PGE₂, PGF₂ α , and PGD₂ (Harper et al., 1983; Siler-Khodr et al., 1986a). Much like cytokines, they play a role in trophoblast implantation (Lewis, 1982) and in parturition (Challis and Patrick, 1980; Casey and MacDonald, 1988). After implantation, these factors, particularly prostacyclin (PGI₂) and PGE₂, appear to be vasoregulators of the fetal–placental unit (Challis and Patrick, 1980; Ylikorkala et al., 1983; Sorem and Siler-Khodr,

1995). PGI₂ is a potent vasodilator in placental vessels, an inhibitor of platelet aggregation, and a uterine relaxing factor; its loss has been implicated in PE. TXA₂ opposes PGI₂, and production is increased in PE; low-dose aspirin preferentially inhibits TXAs in the placenta and may decrease development of PE.

Immunologic Function

Throughout pregnancy the risk of fetal infection must be balanced against fetal rejection. This balance is maintained on both the maternal and fetal sides of the placenta. Unique features of the cells at the placental interface are required to allow the genetically distinct fetal “graft” to inhabit the maternal host. Placental trophoblast cells directly encounter maternal immune cells: SynT_s covering the placental villi are bathed in maternal blood and the invading trophoblasts exposed to the maternal decidua. Different strategies appear to be used at these sites to prevent destruction by cytotoxic maternal immune cells. For example, neither SynT_s nor invading trophoblasts express classic human leukocyte antigen (HLA)-A or HLA-B class Ia major histocompatibility complex antigens nor HLA class II antigens. However, invading trophoblasts do express nonclassic HLA-G and HLA-C, HLA types that can actually suppress immune responses, especially through leukocyte inhibitory receptors on uterine natural killer (NK) cells and macrophages. A balance of innate immunity and modulation of adaptive immune responses is required, and this balance shifts throughout gestation (Hunt et al., 2010; Christiansen, 2013).

The decidua is replete with innate immune cells including T cells, regulatory T cells, macrophages, dendritic cells, and uterine NK (uNK) cells. The best-studied subtype is the NK population. NK cells peak and constitute the largest leukocyte population in the early pregnant uterus, accounting for 60%–70% of total lymphocytes. These cells diminish in proportion as pregnancy proceeds. Despite being replete with cytotoxic perforin, granzymes A and B, and the natural cytotoxicity receptors (NKp30, NKp44, NKp46, NKG2D, NGK2B4, and LFA-1), these NK cells are tolerant cytokine-producing cells at the maternal–fetal interface (Kalkunte et al., 2008). The temporal occurrence around the SpAs and timed amplification of these specialized NK cells observed during the first trimester implicate their role in SpA remodeling. NK cell-deficient mice display abnormalities in decidual artery remodeling and trophoblast invasion, possibly because of a lack of uNK cell-derived interferon γ (Ashkar et al., 2000). Other studies have shown that uNK cells are a major source of VEGF-C, angiopoietins 1 and 2, and TGF- β 1 within the placental bed that decrease with gestational age (Lash et al., 2006). These observations implicate uNK cells in promoting angiogenesis. Recent studies suggest that VEGF-C may induce the noncytotoxic activity in maternal immune cells as well (Kalkunte et al., 2009). Additional molecules expressed on trophoblasts, such as members of the B7 family that alter lymphocyte activity and FasL, which interacts with Fas leukocyte receptors, may also modulate cytotoxicity in the placenta.

Both maternal macrophages and Hofbauer cells (macrophages in the villi that are derived from the fetus) are present in the placenta during pregnancy. These cells may prevent uterine infections or facilitate vascular remodeling and immune suppression (Nagamatsu and Schust, 2010b). Much like NK populations, alterations in macrophage activation, both maternal and fetal, have been linked to pregnancy complications such as IUGR, preterm birth, and PE (Nagamatsu and Schust, 2010a).

Maternal tolerance to fetal alloantigens was initially explored in the context of T-helper cells (Th)1/Th2 balance in mice, with

Th2 cells and cytokines proposed to predominate over Th1 cellular immune response under normal pregnancy. In human pregnancy, the role of specialized T lymphocytes, termed *regulatory T cells* (Tregs), in producing immune tolerance has emerged. Tregs are potent suppressors of T-cell-mediated inflammatory immune responses and prevent autoimmunity and allograft rejection. CD4+CD25+ Tregs are found in the decidua throughout pregnancy. Fetal-specific Tregs persist between pregnancies, and they accumulate and reexpand their population rapidly in subsequent pregnancies, potentially providing a persistent protective regulatory memory to fetal antigen (Rowe et al., 2012). The specific role of these Tregs in human pregnancy loss remains to be defined.

Immunosuppressive immune modulators are also highly expressed at the placental interface. Many of the endocrine factors produced by the placenta (progesterone, PGE2, and interleukins) as described above appear critical to maternal immune modulation. For example, the antiinflammatory cytokine interleukin (IL)-10 is expressed by human trophoblasts and Tregs, increasing across the first two trimesters and then declining before delivery. Low IL-10 expression has been linked to pregnancy loss and preterm delivery, as well as PE. However, how IL-10 protects the fetus is poorly understood. IL-10^{-/-} mice are fertile if maintained pathogen-free but are highly susceptible to complications from infection suggesting that IL-10 deficiency plus a “second hit” such as infection, environmental factors, or hormonal dysregulation may contribute to poor pregnancy outcomes (Thaxton and Sharma, 2010).

There is no generalized immunosuppression in pregnant women. Rather, there is a balance struck between specific types of immune suppression and activation. Indeed, cytokine production capacity is higher in pregnant than in nonpregnant women. It is the balance of proinflammatory to antiinflammatory cytokines that may determine outcome. When this balance is altered early in gestation, implantation could be affected, while immune alterations in late gestation may contribute to susceptibility to preterm birth, particularly in the face of an immune challenge. Similar shifts in the fetal immune response from tolerance to activation are being investigated across normal gestation (Mold et al., 2010). The role of placental immune activation in poor neonatal outcome, particularly in neurologic complications, has become an area of active study in the past decade (Kim et al., 2015a; Kim et al., 2015b). Defining and manipulating placental immune responses are key components of current efforts to improve pregnancy outcomes.

Fetal Programming

Complex yet intricate interactions between maternal and fetal systems promote fetal growth and normal pregnancy outcomes, interactions that must occur via the placenta. The placenta does not play a passive role as the interface between mother and fetus. Rather, it adapts to maternal status—nutrition, stress, environmental exposures—with altered vascularity and cellular composition and changes in endocrine and transport functions. Fetal genotype and fetal metabolic demands can also alter placental nutrient transfer and possibly other placental functions. Epidemiologic evidence first suggested that these complex interacting pathways alter the in utero environment in ways that lead to long-term changes in health and disease, particularly cardiovascular and metabolic disease (Barker and Osmond, 1986). How these interactions occur, as well as specific links between the in utero environment and later adult morbidities, is referred to as “fetal programming” or the “developmental origins of health and disease” (Fig. 5.5) (Burton et al., 2016).

Nutrition is the best-studied mechanism of fetal programming, although many other maternal and fetal programming events likely have long-term impacts (Fig. 5.6). Undernutrition can elicit placental and fetal adaptive responses that lead to local ischemia and metabolic, hormonal, and immune reprogramming, resulting in small for gestational age (SGA) fetuses. Maternal health and dietary status, exposure to environmental factors, uteroplacental blood flow, placental transfer, and fetal genetic and epigenetic responses likely all contribute to in utero fetal programming. Adult diseases such as coronary heart disorders, hypertension, atherosclerosis, type 2 diabetes, insulin resistance, respiratory distress, altered cell-mediated immunity, cancer, and even psychiatric disorders are being linked to fetal programming in utero (Sallout and Walker, 2003). In addition to maternal predisposing factors, cytokines, hormones, growth factors, and the intrauterine immune milieu also contribute to in utero programming. Therefore a healthy mother with healthy placentation is critical to healthy fetal outcomes.

Regulation of Placental Function

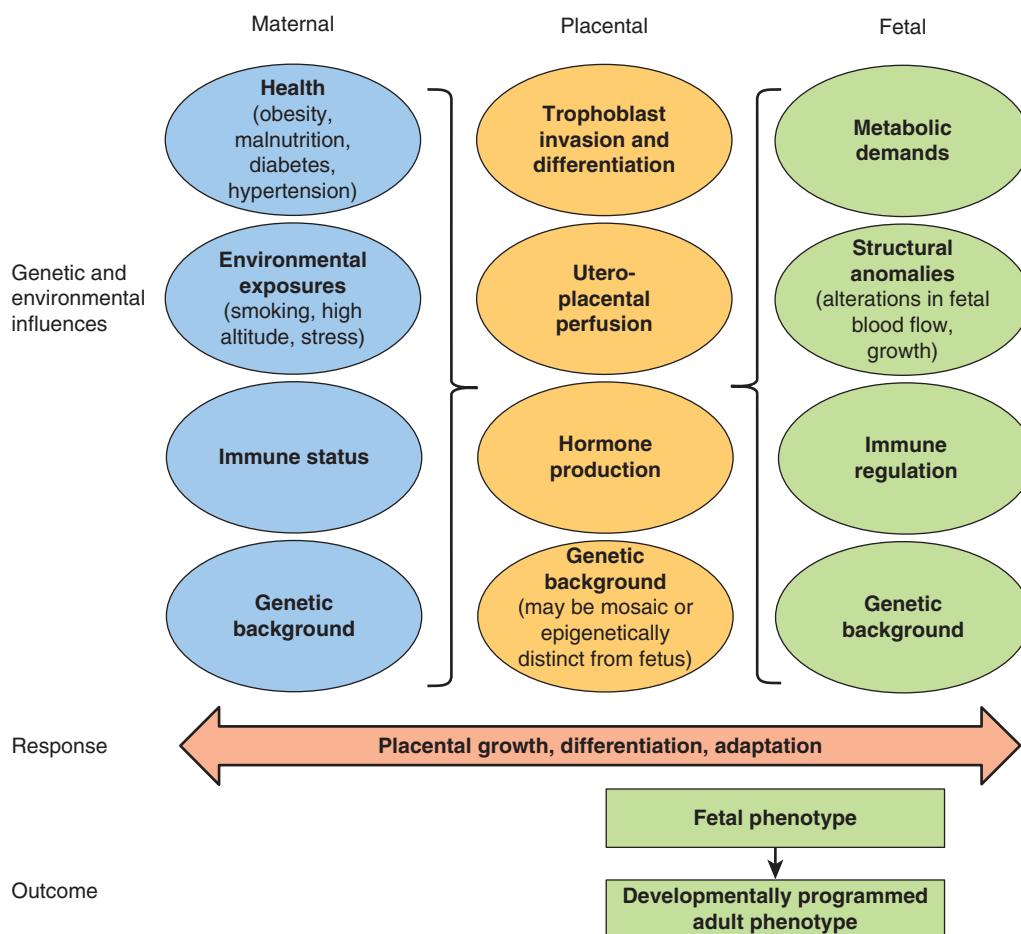
While it is now recognized that placental function can have lifelong impacts on health, the molecular mechanisms that underlie this complex signal integration and regulation are not yet well understood but are critically important to human disease. An understanding of the molecular mechanisms underlying the placental integration of maternal and fetal information that results in fetal programming may suggest new ways to manage pregnancy complications that lead to fetal growth failure and long-term alterations to health. To date, the two best documented forms of placental regulation are (1) signal integration thorough specific signaling cascades and (2) epigenetic modifications of placental gene expression.

Nutrient-Sensing Signaling Pathways

Multiple signaling molecules are expressed in SynT cells that are responsive to nutrient supply, including mechanistic target of rapamycin (mTOR), adenosine monophosphate-activated protein kinase, and glycogen synthase-3 (see Fig. 5.6). Emerging evidence suggests that mTOR may be a key component of the placental nutrient-sensing signaling pathway (Jansson and Powell, 2013; Dimasuay et al., 2016). mTOR is a serine/threonine-specific protein kinase belonging to the family of phosphatidylinositol-3 kinase (PI3K)-related kinases. mTOR regulates cellular metabolism, growth, and proliferation by targeting and signaling through two protein complexes, mammalian target of rapamycin complex (mTORC1) and mTORC2. Both hypoxia and limited nutrient supply result in the mTORC1 inhibition seen in placentas of IUGR fetuses (Roos et al., 2007; Kavitha et al., 2014). In the case of maternal obesity and enhanced nutrition, mTORC1 appears to be activated. mTOR expression in turn regulates nutrient transporter trafficking, particularly amino acid transporters. Recent experiments in mice suggest that the maternal and the fetal genotype of the upstream regulator of mTOR, PI3K, both influence placental function, nutrient delivery, and maternal physiology (Sferruzzi-Perri et al., 2016). While the regulatory details of this pathway need to be elucidated in humans, regulating components of the mTOR pathway might provide novel treatments for disorders of fetal growth (Jansson and Powell, 2007).

Epigenetic Regulation in the Placenta

The placenta has unique epigenetic features that may make it particularly responsive to its environment. Epigenetic regulation



• **Fig. 5.5** Fetal Programming. Maternal and fetal health alter placental function, which in turn influences fetal adaptations. Dietary status, exposure to environmental factors, uteroplacental blood flow, placental transfer, and genetic and epigenetic changes contribute to in utero fetal programming.

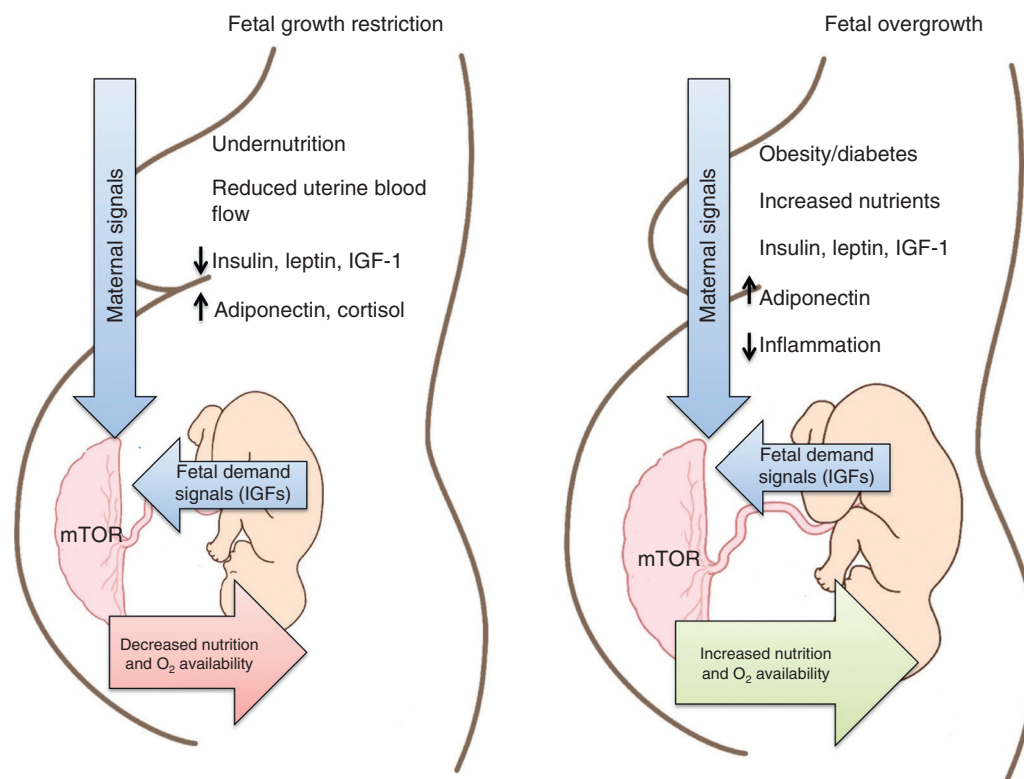
of gene expression allows for heritable changes that are mediated independently of changes in DNA sequences themselves. Gene expression is largely determined by the accessibility of DNA to transcription factors, accessibility that is controlled by a variety of epigenetic mechanisms: methylation of DNA promoter regions, histone modifications, genomic imprinting, and expression of noncoding ribonucleic acids (RNAs) such as microRNA (miRNA). Since these alterations are not encoded in DNA, epigenetic changes can occur in response to the cellular environment. In utero changes in the epigenome have been linked to poor pregnancy outcomes, although the causal relationships remain unclear (Januar et al., 2015).

Massive epigenetic reprogramming with loss of DNA methylation occurs just after fertilization and before implantation. The placenta and other extraembryonic tissue remain hypomethylated, even though methylation increases with cellular differentiation so that even at term, the placenta is the most hypomethylated human tissue (Bianco-Miotto et al., 2016). Within the placenta, the various cell types have distinct methylation profiles. Nutritional components, such as folate, can alter placental and fetal methylation patterns (Jansson and Powell, 2007). Placental methylation changes have been linked to PE, IUGR, and preterm birth (Januar et al., 2015; Bianco-Miotto et al., 2016).

Methylation changes also contribute to genomic imprinting, in which an allele is silenced in a specific parent-of-origin manner.

Placental genomic imprinting is distinct from somatic imprinting, with most imprinted placental genes impacting fetal and/or placental growth or maternal preparation for care of offspring. Most of what is known about placental gene imprinting comes from studies on the IGF pathway in mouse, described above. Paternally expressed imprinted genes, such as *IGF2*, promote placental and fetal growth while maternally expressed ones, such as *IGF-2R*, suppress growth (Gicquel and Le Bouc, 2006). However, recent human studies suggest significant species specificity of imprinting, emphasizing the need for large-scale human placental studies to better define the role of this epigenetic mechanism in human pregnancy (Monk, 2015).

An additional layer of epigenetic regulation may be added by miRNAs, the small noncoding RNA gene products that regulate gene expression through repression of messenger RNA (mRNA) translation or mRNA decay (Mouillet et al., 2015). At least 500 miRNA species are expressed in the placenta, and those expressed in trophoblasts can be detected in maternal circulation (Chim et al., 2008) (Fig. 5.7). The functions of some of these miRNAs are starting to be decoded. For example, miR-675 is an miRNA embedded in the first exon of H19, a highly expressed large intergenic noncoding RNA. miR-675 is expressed only in the placenta when placental growth is slowing in the second half of gestation. Loss of H19 and thus miR-675 results in placental overgrowth, while overexpression of the miRNA results in reduced proliferation (Keniry et al., 2012).



• **Fig. 5.6** Placenta as a Nutrient Sensor. The role of the placenta in maternal–fetal resource allocation has been hypothesized to be a critical element of fetal programming (Jansson et al., 2013; Sferruzzi-Perri and Camm, 2016). Maternal and fetal genotype influence signaling pathways (i.e., the phosphoinositol 3-kinase/mechanistic target of rapamycin [*mTOR*] pathway) in the placenta that balance maternal environmental status and fetal demands. When the maternal nutrient supply is altered, the placenta responds to maternal signals to match fetal growth to the nutrient supply. The placental alterations are thus central to the long-term health of the fetus. *IGFs*, Insulin-like growth factors; *IGF-1*, insulin-like growth factor-1.

It is notable that epigenetic mechanisms used by the placenta are most similar to those seen in tumorigenesis, reflecting their invasive similarities. Large-scale patterns of hypomethylation, as well as site-specific hypermethylation of tumor suppressor genes often seen in cancers, occur in the placenta. In addition, some miRNAs that have been implicated in regulating uterine invasion are associated with malignancies when reactivated outside of the placental context (Mouillet et al., 2015). Given the limited invasive potential of the placenta under normal circumstances, there is likely to be an additional layer of regulation controlling the placental epigenetic response to both intrinsic and environmental cues.

Placental Diseases

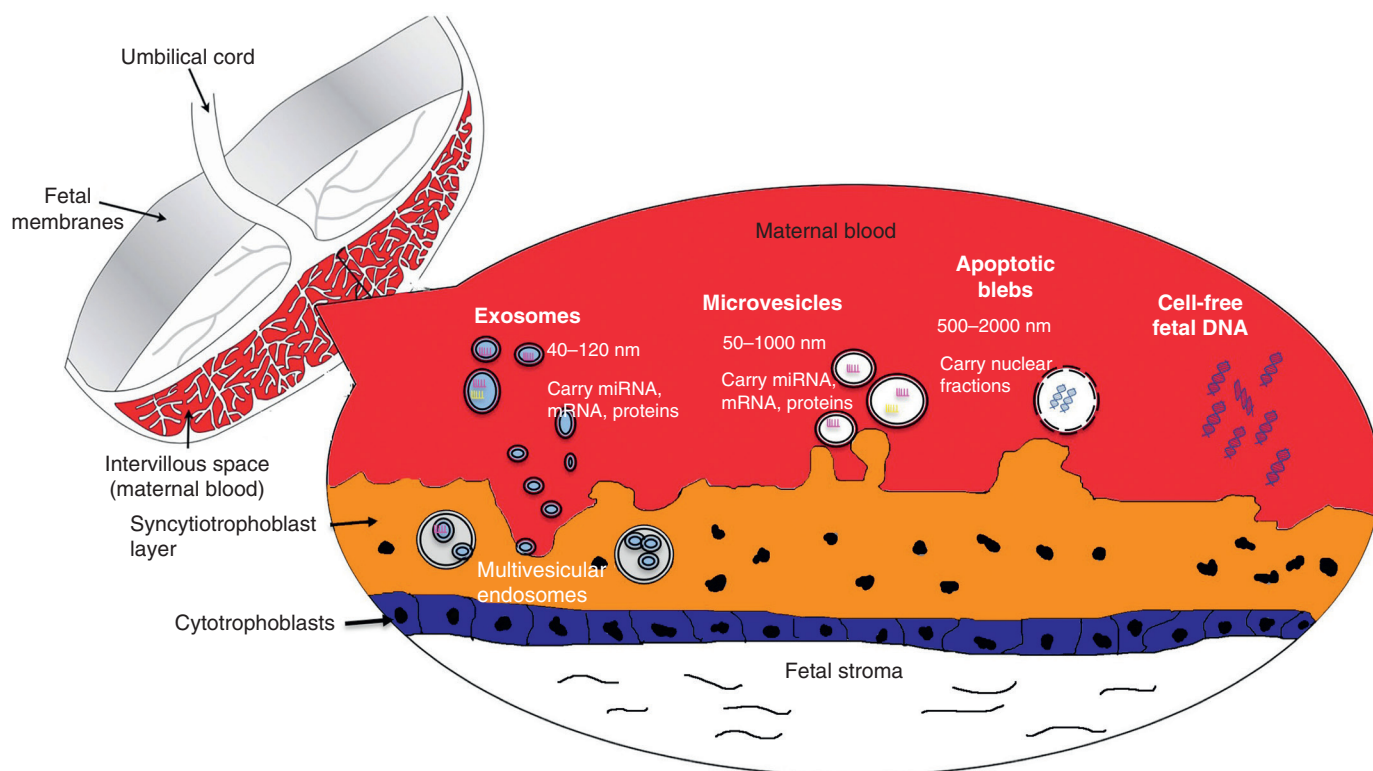
Understanding placental dysfunction and disease during pregnancy is critical to improving neonatal outcomes. Healthy development of the placenta requires efficient metabolic, immune, hormonal, and vascular adaptation by the maternal system as well as the fetus. Abnormal placentation and placental infections can lead to PE, growth retardation, or preterm birth, which can have a lifelong bearing on health. Most major obstetric syndromes originate in early gestation because of abnormal trophoblast invasion or immune dysregulation but present clinically in late gestation. Maternal factors such as ascending infections, obesity, hypertension, diabetes, and environmental exposures also contribute to placental dysfunction.

Placental Disorders of Pregnancy

Preeclampsia

Maternal hypertension affects 5%–10% of human pregnancies, mainly due to PE (Ghulmiyyah and Sibai, 2012). PE is clinically associated with maternal symptoms of hypertension, proteinuria, and glomeruloendotheliosis; it can progress to eclampsia resulting in seizure, coma, and maternal death. The most common fetal complication is growth restriction (Srinivas et al., 2009). Early onset (<24 weeks' gestation) carries a greatly increased risk of IUGR. PE resolves only with delivery of the fetus and placenta. The maternal symptoms are mediated mainly by secreted placental factors, while the fetal symptoms result from impaired placental perfusion. Abnormal remodeling of SpAs and shallow trophoblast invasion are two hallmark features of PE (Brosens et al., 2011), but the etiology of this failure to invade remains unclear; endothelial dysfunction is a key component, and both fetal and maternal factors are thought to contribute. Poor placental perfusion may cause release of circulating factors leading to maternal symptoms, which then exacerbate placental failure and impaired fetal growth.

Despite limited mechanistic understanding of placental pathology leading to PE, several pathways are consistently implicated in this disease. Angiogenic and antiangiogenic factors, excessive complement cascade activation, and immune intolerance may all play a role. In normal pregnancy, angiogenic factors including VEGF and circulating PlGF steadily increase in the first and second trimesters,



• **Fig. 5.7** Placental Products Circulate in Maternal Blood During Gestation. The placental syncytiotrophoblasts produce and secrete extracellular vesicles of various sizes, shapes, and functions into the maternal circulation in both healthy and compromised pregnancies. The function and cargo of these vesicles is not yet completely defined but likely plays a role in maternal–fetal cross talk between cells during pregnancy. Circulating placental products may also provide biomarkers for placental function and pathologies. Exosomes are constitutively secreted products of the endosomal pathway that carry proteins, micro ribonucleic acid (RNA) and messenger RNA that can modulate maternal cell functions, including immune and endothelial responses. Microvesicles and apoptotic bodies are larger vesicles produced by direct budding of the plasma membrane following alterations in cellular conditions (oxygen tension, glucose, or calcium level changes) or as part of apoptosis, respectively. These vesicles may carry unsorted protein and nucleic acid cargo picked up from the cell cytosol. They may have a proinflammatory role in pregnancy. Circulatory cell-free DNA can be up to 13% of cell-free DNA in maternal circulation. Circulatory cell-free DNA is fragmented and significantly smaller than maternal fragments, allowing fragment size and sequence to be used to distinguish fetal from maternal origin. *mRNA*, Messenger ribonucleic acid; *miRNA*, micro RNA; *nm*, nanometers.

peak at 29 to 32 weeks, and decline thereafter. However, free VEGF remains low and unchanged during this window. Reduced placental expression of VEGF and PlGF is consistently observed in PE. Furthermore, PE is frequently accompanied by enhanced placental expression and free circulation of the soluble fms-like tyrosine kinase-1 (sFlt-1), which binds to and inactivates VEGF and PlGF (Levine et al., 2004a; Romero et al., 2008). Soluble endoglin, which enhances sFlt-1 activity, is also elevated in PE (Levine et al., 2004a; Venkatesha et al., 2006). Excessive complement cascade activation and immune activation may also contribute to PE, although whether it is a cause or effect of the pathology is unclear (Lynch et al., 2008). Altered regulatory T-cell function may alter trophoblast interaction with the uterine lining, and increased villous turnover from placental damage may increase maternal immune system response to circulating placental debris (Laresgoiti-Servitje et al., 2010).

Intrauterine Growth Restriction

IUGR or fetal growth restriction designates a fetus that has not reached its growth potential; it can be caused by fetal, placental, or maternal factors. Disparities between fetal nutritional or

respiratory demands and placental supply can result in impaired fetal growth. PE is a frequent cause of IUGR, but placental surface area reduction with decreased villi and fetal capillaries can be seen in IUGR alone, suggesting distinctive disease etiologies (Daayana et al., 2004; Srinivas et al., 2009). Chromosomal abnormalities (aneuploidy, partial deletions, and gene mutation, particularly on the gene for IGFs), congenital abnormalities, multiple gestation, and infections can also result in IUGR. IUGR may result in an SGA newborn. Mortality and morbidity are increased in SGA infants compared with those who are appropriate for gestational age. At birth, SGA infants may have impaired thermoregulation; poor cardiopulmonary transition with perinatal asphyxia, pulmonary hypertension, hypoglycemia, polycythemia, and hyperviscosity; impaired cellular immune function; and increased risk for perinatal mortality. In childhood, having been SGA increases the risk of neurodevelopmental impairments, and in adulthood, cardiovascular and metabolic disease risks are elevated (Sallout and Walker, 2003).

Preterm Birth

Infants born before the 37th week of gestation are considered premature, and their care places an enormous burden on the

healthcare infrastructure. Preterm infants face an increased risk of lifelong disabilities such as mental retardation, learning and behavioral problems, autism, cerebral palsy, chronic lung diseases, vision and hearing loss, and an increased risk for diabetes, hypertension, and heart disease in adulthood. In countries such as the United States, preterm birth accounts for approximately 10%–13% of all deliveries (Goldenberg et al., 2008). Despite improvements in our understanding of the risk factors associated with preterm delivery, the rate of prematurity has risen over the past two decades, due in large part to an increase in the rate of indicated preterm deliveries (Goldenberg et al., 2008). In the United States, iatrogenic delivery is responsible for almost half the births that occur between 28 and 37 weeks of gestation, primarily because of placental pathologies such as PE or IUGR. The majority of spontaneous preterm deliveries are due to preterm labor. Other factors leading to spontaneous premature birth are preterm premature rupture of membranes, cervical incompetence, and antepartum bleeding. Additional risk factors for preterm birth include stress, occupational fatigue, uterine distention by polyhydramnios or multifetal gestation, systemic infection such as periodontal disease, intrauterine placental pathology such as abruption, vaginal bleeding, smoking, substance abuse, maternal age (<18 or >40 years), obesity, diabetes, thrombophilia, ethnicity, anemia, and fetal factors such as congenital anomalies and growth restriction.

There is increasing evidence that approximately 50% of preterm births are associated with infection of the decidua, amnion, or chorion and amniotic fluid caused by either systemic or ascending genital tract infection (Goldenberg et al., 2008). Both clinical and subclinical chorioamnionitis are implicated in preterm birth. Maternal or fetal inflammatory responses to chorioamnionitis infection can trigger preterm birth (Romero et al., 2014). Activated neutrophils and macrophages and the release of cytokines IL-1 β , IL-6, IL-8, TNF- α , and G-CSF can lead to an enhanced cascade of signaling activity, causing release of PGs and expression of various MMPs of fetal membranes and the cervix. Elevated levels of TNF- α and apoptosis are associated with term premature rupture of membranes. Noninfection-related inflammation caused by placental insufficiency and apoptosis can also cause preterm birth. In addition to augmented inflammatory responses to infections, pathogenic microbes (e.g., *Staphylococcus*, *Streptococcus*, *Bacteroides*, and *Pseudomonas* spp.) are thought to directly degrade fetal membranes by releasing proteases, collagenases, and elastases, producing phospholipase A2, and releasing endotoxin that stimulates uterine contractions and causes preterm birth (Slattery and Morrison, 2002).

Interaction between the maternal HPA axis as a result of major maternal physical or psychological stress may also alter the normal placental production of HPA-like hormones, including increased production of CRH, which leads to increased fetal ACTH and cortisol production. Premature activation of the fetal HPA axis can eventually stimulate PG production, ultimately resulting in parturition. In addition, activation of the HPA axis promotes increases in estrogens, decreased progesterone, and OTR expression, further enhancing myometrial activation and preterm birth (Jones and Challis, 1989; Mesiano and Jaffe, 1997).

Gestational Diabetes

Impaired maternal glucose tolerance during pregnancy results in GDM. Impaired placental function may alter hCS production, and in turn, hyperglycemia can impair placental development, growth, and nutrient transport (Araujo et al., 2015). Remarkably, GDM can be associated with either IUGR or, more commonly, with fetal overgrowth (Sibley et al., 2005; Jansson et al., 2006). Placental integration of multiple signals including glucose levels,

intrinsic genetic susceptibility, and fetal demands may be a critical regulator of this fetal growth outcome (Sibley et al., 2005).

Central Nervous System Injury

Both acute and chronic placental dysfunction are linked to adverse neurologic outcomes in fetuses and neonates. Acute mechanical disruptions of placental function (umbilical cord occlusion or placental abruption) can result in severe neurologic hypoxic–ischemic damage before or during delivery but are relatively rare events. More commonly, chronic placental vascular lesions (chronic villitis, fetal thrombotic vasculopathy, or infection-associated fetal vasculitis) are correlated with neurologic injury, including cerebral palsy (Redline, 2005). Almost 90% of term infants with neonatal encephalopathy and brain injury have placental lesions noted postdelivery (Wintermark et al., 2010), although the high frequency of placental lesions in apparently neurologically intact newborns makes interpretation complex (Lachapelle et al., 2015; Redline, 2015). Preterm birth is an independent risk factor for neurologic injury, but chorioamnionitis, which frequently causes preterm delivery, is also associated with brain injury and increased risk of cerebral palsy, particularly when funisitis or severe fetal inflammation is present (Leviton et al., 1999; Romero et al., 2014; Kim et al., 2015a). IUGR infants are also at significantly increased risk of neurodevelopmental impairments (Redline et al., 2007; Apel-Sarid et al., 2010).

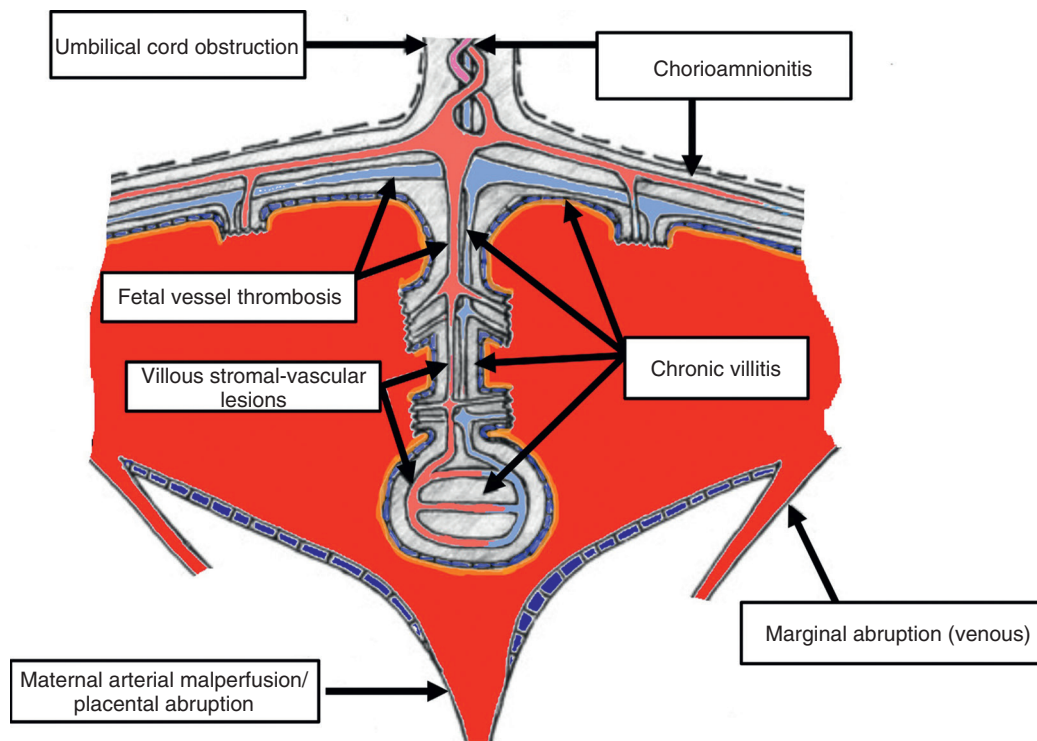
Evaluation of Placental Dysfunction

Until recently, placental assessment was performed almost exclusively after delivery, using traditional anatomic pathology techniques. While significant correlations have been described between specific pathologic lesions and neonatal outcomes, our understanding of how and when these lesions develop during gestation and ultimately lead to poor outcomes remains limited. There has been a recent resurgence of interest in developing advanced tools to investigate placental function during gestation, particularly following the launch of the “Human Placenta Project” by the Eunice Kennedy Shriver National Institutes of Child Health and Disease (Guttmacher et al., 2014; Guttmacher and Spong, 2015). In addition to promoting the development of new advanced imaging techniques (Andescavage et al., 2015; Siauve et al., 2015) and biomarker methods for measuring placental function (Cuffe et al., 2017), there has been a renewed interest in standardizing placental histopathologic classification and diagnosis. In 2014 an international group of placental pathologists met in Amsterdam to establish consensus guidelines for placental examination and classification of lesions (Khong et al., 2016), and these are summarized in Table 5.1.

Placental Histopathology

Direct examination of the placenta after birth can give some clues to the timing and extent of important adverse prenatal or neonatal events. Some disorders are readily apparent in the delivery room (listeria lesions, placental abruption associated with large clots, abnormal cord insertion), and others require more detailed gross and microscopic examination. In a high-risk delivery service, approximately 50% of placentas qualify for pathologic examination (Curtin et al., 2007).

The majority of placental lesions described by pathologists involve either vascular or immunologic/infectious processes (Table 5.1). Vascular processes can be further localized as maternal or fetal lesions (Fig. 5.8, villous maternal stromal-vascular lesions versus fetal-vascular lesions), potentially providing clues to both the underlying etiology of the lesion and its implications for maternal



• **Fig. 5.8** Histopathologic Sites of Action of Major Placental Disease Processes. (Modified from Redline RW. The clinical implications of placental diagnoses. *Semin Perinatol.* 2015;39:2–8.)

or fetal death. Immune processes likewise can be subdivided into chronic or acute infections and also distinguished from inflammatory processes associated with immune activation without infection (see Fig. 5.8, chronic villitis). Links have been made between each major pathologic lesion type and different pregnancy complications (Table 5.2).

Placental Imaging

Ultrasound (US) imaging remains the standard imaging method used for placental evaluation during pregnancy because of its availability, safety, and relatively low cost. However, US evaluation of placental anatomy can be limited by placental implantation site, maternal body habitus, and amniotic fluid volume, and US-detected lesions correlate poorly with postnatal histopathology (Moran et al., 2011). As a primary screening tool, placental US has proven very useful, but its use for functional placental assessment that can predict which pregnancies are at risk of later placental compromise is limited.

The placenta may be visible on US as early as 5 weeks' gestation (Wong et al., 2009). It becomes readily visible by 15 weeks' gestation by US and undergoes progressive increases in thickness and diameter as well as changes in echogenicity and shape (Moran et al., 2011). Both increased and decreased placental size are associated with abnormal development and risk of fetal complications. Many placental lesions can be seen by US as pregnancy progresses. Some, such as placental lakes, which are anechoic regions of low maternal blood flow, are very common but appear to be of limited clinical significance. Others, including echogenic areas of infarct, may be significant when large or centrally located. Placental calcifications increase across gestation. There appears to be an association between early development of calcifications and poor placental function, but use of early, high-grade calcification to predict pregnancy outcome has proved unreliable (Moran et al., 2011). Doppler

velocimetry is used in conjunction with anatomic US to functionally assess placental blood flow (Abramowicz and Sheiner, 2008). Fetal villous vascular damage results in high resistance in the umbilical artery (UA) circulation, and chronic fetal hypoxia decreases umbilical venous flow. Loss of UA end-diastolic flow is associated with severe IUGR and indicative of significant fetal compromise. Monitoring of placental blood flow allows detection of high resistance and poor circulation within the placenta but is usually apparent only when significant fetal compromise has already occurred. Additional US measures that may be more predictive of placental compromise, such as 3-dimensional placental volumes and vascularization indices (Moran et al., 2011), as well as new elastography and higher resolution US techniques, are under investigation.

Magnetic resonance imaging (MRI) is increasingly being used for anatomic placental evaluation, and advanced functional techniques may yield information on oxygenation, vascularization, and metabolism (Andescavage et al., 2015; Siauve et al., 2015). MRI benefits from having multiplanar images in a wider field of view, as well as having higher spatial and temporal resolution when compared with US. It is currently used primarily to assess fetal structural anomalies and, more recently, for improved detection of placenta accreta and other invasive placental anomalies (Lam et al., 2002). Precise quantitation of placental volume is more readily performed using MRI than US and may allow earlier prediction of fetal growth retardation based on small placental size (Derwig et al., 2011; Andescavage et al., 2015). Use of standard MRI gadolinium-based contrast agents has been limited due to fetal safety concerns, but new agents are being developed. Placental application of noncontrast functional MRI methods, including diffusion-weighted imaging and diffusion tensor imaging, are being investigated along with methods that rely on endogenous contrast agents, such as hemoglobin-based detection of oxygen level changes measured by blood-oxygen level-dependent MRI (Andescavage

TABLE 5.2 Common Placental Causes of Specific Adverse Pregnancy Outcomes

1. Fetal Growth Restriction
Maternal stromal-vascular lesions: global/partial vascular malperfusion with accelerated villous maturation
Fetal stromal-vascular lesions: developmental lesions (superficial implantation), global/partial vascular malperfusion (fetal thrombotic vasculopathy)
Villitis of unknown etiology
2. Spontaneous Preterm Birth
Maternal inflammatory response: acute chorioamnionitis
Maternal vascular lesions: mild global/partial vascular malperfusion with accelerated villous maturation
Acute marginal placental abruption
3. Preterm Fetal Death
Maternal stromal-vascular lesions: global/partial vascular malperfusion with accelerated villous maturation
Fetal global/partial vascular malperfusion (cord accidents/obstruction)
Placental abruption
4. Term Fetal Death
Fetal stromal-vascular lesions: developmental lesions (delayed villous maturation), global/partial vascular malperfusion (cord accidents/obstruction)
Placental abruption
Fetomaternal hemorrhage
5. Term Neurologic Injury
Fetal stromal-vascular lesions: global/partial vascular malperfusion (fetal thrombotic vasculopathy or cord occlusion)
Chronic villitis of unknown etiology with obliterative fetal vasculopathy
Acute chorioamnionitis with severe fetal inflammatory response
Presence of multiple placental lesions

Modified from Redline RW. Classification of placental lesions. *Am J Obstet Gynecol*. 2015 Oct;213(4 Suppl):S21-8.

et al., 2015; Siauve et al., 2015). Use of placental MRI for real-time assessments is likely to provide new information about placental vascular development and function, as well as new diagnostic tools for use in high-risk pregnancies.

Serum Biomarkers of Placental Disease

A major goal of pregnancy screening is to identify women early in gestation who will go on to develop placenta-mediated complications that threaten either fetal or maternal health so that targeted early therapies can be provided. Detection of factors released by the placenta into maternal circulation that predict disease is a longstanding area of investigation. In addition to physiologic secretion of placental factors into maternal circulation, cellular stress (i.e., oxidative, hypoxic, or inflammatory stress) can lead to increased villous trophoblast turnover with release of placental vesicles and cellular debris into the circulation (Cuffe et al., 2017). Maternal serum analytes, circulating cell-free DNA, and extracellular vesicle contents derived from the placenta are under investigation as potential biomarkers of placental dysfunction.

Serum Analytes

Maternal serum screening has been applied successfully in the identification of fetuses at increased risk of aneuploidy or structural anomalies (open neural tube defects, abdominal wall defects). The association is less clear between pregnancies at risk of placental dysfunction and abnormal values for the most common first and

second trimester serum screening markers: alpha fetoprotein, hCG, unconjugated estriol (uE₃), inhibin-A, and pregnancy-associated plasma protein-A (PAPP-A). Both single serum analyte abnormalities and combinations have been assessed for their value as biomarkers of specific pregnancy complications, with limited success (Gagnon et al., 2008). For example, higher levels of second trimester inhibin-A levels are associated with PE, although no predictive cutoff level has been identified. Elevated inhibin-A in combination with UA Doppler abnormality, however, may be strongly predictive of PE (Ay et al., 2005). Likewise, extensive studies of low PPAP-A and UA Doppler changes suggest an association with fetal growth restriction and PE (Pilalis et al., 2007). Some alterations of these maternal serum markers have been associated with specific pathologies linked to poor placental function, but the strongest associations are serum marker abnormalities and a generalized increased risk of third trimester fetal death (Gagnon et al., 2008). Increased surveillance or treatment of these pregnancies has not yet shown clinical benefit.

Additional maternal serum biomarkers of placental dysfunction, particularly in PE, include elevated circulating sFlt-1 and reduced PlGF. Syncytial stress leads to placental secretion of these and other angiogenic factors (Cuffe et al., 2017). A recent metaanalysis suggested that the diagnostic accuracy of maternal sFlt-1/PlGF for early onset PE is high, but false positives and false negatives are both greater than 15%, limiting the utility of this ratio as a broad clinical screening tool (Liu et al., 2015).

Circulating Cell-Free Fetal DNA

Direct measurement of DNA found in maternal serum has become possible in the past decade. Continuous turnover of placental villous trophoblasts releases placental microparticles and freely circulating nucleic acids (see Fig. 5.7). These fragments are called fetal but actually originate from the placenta (Taglauer et al., 2014). Noninvasive prenatal screening for aneuploidies and genetic mutations using circulating cell-free fetal DNA (cffDNA) screening from maternal serum has rapidly gained popularity in the past 5 years. Confined placental mosaicism with a normal fetal karyotype can confound these screening results, but the high sensitivity and specificity of these tests combined with their limited risk compared with chorionic villous sampling or amniocentesis have led to their rapid clinical adoption. Total cffDNA levels, rather than specific cffDNAs, may also be useful biomarkers for placental health and function. cffDNA is increased in PE, both before and during the development of clinical symptoms, likely because of increased trophoblast apoptosis associated with oxidative stress (Levine et al., 2004b). High concentrations of cffDNA are also associated with increased preterm birth risk (Farina et al., 2005). Cell-free RNA and miRNAs have also been found in maternal circulation, and their utility as biomarkers is actively being assessed.

Extracellular Vesicles

Multiple types and sizes of vesicles are shed by the placenta in both normal and compromised pregnancies (see Fig. 5.7), but the amounts and contents vary with placental health (Cuffe et al., 2017). Exosomes are a subtype of extracellular vesicle derived from endosomes that carry proteins and RNAs and that are released by exocytosis into the extracellular space. Exosomes play a significant role in intercellular signaling in multiple systems, and their role in pregnancy has garnered intense interest in the past few years (Mitchell et al., 2015). Placentally derived exosomes carry SynT specific proteins, including placental alkaline phosphatase and the miRNAs from H19 described previously, allowing their identification as placental vesicles in maternal circulation. Their release is regulated

by multiple environmental factors including oxygen tension and glucose concentrations, making them particularly appealing as reporters of placental function. Exosomes mediate communication between the placenta and maternal immune cells, and widespread placental–maternal cellular communication using this mechanism has been proposed. There is a general increase in placental exosomes

in maternal circulation across gestation, and these levels may vary with placental pathology; the vesicle contents may also reflect pathology, such as decreased cell fusion proteins in exosomes from preeclamptic placentas (Mitchell et al., 2015). As placental exosome biology becomes better defined, use of exosomes as biomarkers of placental function is an exciting possibility.

Summary

The placenta is a complex organ that develops from many cell types to form a sophisticated interface between mother and fetus that integrates intrinsic and extrinsic signals to optimize fetal development. The consequences of impaired placental function have

lifelong impact not only on individual offspring but potentially on multiple generations, effected by epigenetic changes. Understanding and optimizing placental health is critical, not just to newborns but also to improving health outcomes for the entire population.

Suggested Readings

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