Placentation in the human and higher primates

Graham J Burton[1,2] and Eric Jauniaux[3]

[1]Centre for Trophoblast Research, University of Cambridge, Cambridge, UK

[2]Department of Physiology, Development and Neuroscience, University of Cambridge, Cambridge, UK

[3]EGA Institute for Women's Health, Faculty of Population Health Sciences
University College London, London, UK.

Address for correspondence:

Professor G.J. Burton,

Physiological Laboratory,

Downing Street,

Cambridge, CB2 3EG

UK

Email: gjb2@cam.ac.uk

Tel: +44 1223333856

Abstract

Placentation in the human is precocious and highly invasive compared to other mammals. Implantation is interstitial, with the conceptus becoming completely embedded within the endometrium towards the end of the second week post-fertilization. Villi initially form over the entire surface of the chorionic sac, stimulated by histotrophic secretions from the endometrial glands. The secondary yolk sac never makes contact with the chorion, and a choriovitelline placenta is never established. However, recent morphological and transcriptomic analyses suggest the yolk sac plays an important role in uptake of nutrients from the coelomic fluid. Measurements performed in vivo demonstrate that early development takes place in a physiological, low-oxygen environment that protects against teratogenic free radicals and maintains stem cells in a multipotent state. The maternal arterial circulation to the placenta is only fully established around 10-12 weeks of gestation. By then, villi have regressed over the superficial, abembryonic pole, leaving the definitive discoid placenta, which is of the villous, hemochorial type. Remodelling of the maternal spiral arteries is essential to ensure a high-volume but low-velocity inflow into the mature placenta. Extravillous trophoblast cells migrate from anchoring villi and surround the arteries. Their interactions with maternal immune cells release cytokines and proteases that are key to remodelling, and a successful pregnancy.

Introduction

Placentation in the human has long been considered almost unique amongst mammals, involving a precocious and highly invasive form of implantation only seen amongst the great apes. The embedding of the conceptus into the uterine wall was assumed to lead to early establishment of the maternal circulation, which in turn enabled the evolution of our enlarged brain. Over the last two decades these views have been challenged from various directions. Firstly, molecular phylogenetics has revealed that the haemochorial state is likely the ancestral form of placenta and not the derived form once thought (Wildman et al., 2006, Carter and Mess, 2007, Martin, 2008, Elliot and Crespi, 2009). Secondly, despite major morphological differences it has become apparent that many aspects of human placentation are more equivalent to those in other species than previously appreciated. This is particularly true during early pregnancy, for it is now recognised that the human displays an extended period of histotrophic nutrition that continues throughout organogenesis, and that the yolk sac is likely not the vestigial organ it is often portrayed to be. In this chapter we focus on the development of the human placenta, and make comparisons with closely related higher primates.

Origin of the trophoblast lineage

Although the trophoblast is fundamental to the placenta, defining the lineage in molecular terms is problematic, for as yet no exclusive marker has been identified. Instead, it has been proposed that a panel of four criteria be used, including i) selected protein markers such as cytokeratin 7 (KRT7), TF activator protein-2 gamma (TFAP2C) and *GATA*-binding protein 3 (GATA3), ii) expression of microRNAs from the C19 cluster, iii) hypomethylation of the promoter of the transcription factor ELF5, and iv) absence of expression of the HLA class I molecules HLA-A and HLA-B (Lee et al., 2016). Individual components of this panel may feature in different cell types, but collectively they serve to uniquely define the trophoblast.

Activation of the embryonic genome occurs between the 4- and 8-cell stage of development (Braude et al., 1988), and blastocyst formation is usually seen by day 4.5 post-fertilisation (p-f). The epigenetic and transcriptional networks that regulate differentiation into the trophoblast lineage are broadly conserved between the mouse and the human (Hanna et al., 2018), although primate development is protracted compared to that of rodents (Niakan and Eggan, 2013). There are important differences, however. For example, while CDX2, TEAD4, ELF5 and TFAP2C are common to both species, SOX2, ERSB and EOMES are absent in the human (Soncin et al., 2018, Hemberger et al., 2020). Single-cell sequencing of blastocysts has revealed that two sub-populations of trophectoderm cells can be identified by days 6-7 p-f (Petropoulos et al., 2016). The differences in gene expression are mostly related to cell-cell signaling, and consistent with a more differentiated subpopulation localized using immunofluorescence for the chemokine receptor 7 (CCR7) protein to the polar region of the blastocyst overlying the inner cell mass. The receptor for fibroblast growth factor, FGFR1, which is initially expressed on all the trophoblast cells becomes localized to the same region as the blastocyst develops (Niakan and Eggan, 2013).

Implantation

Implantation takes place at ~5-6 days p-f, and as in other species requires a synchronized dialogue between a receptive endometrium and an activated embryo (Teh et al., 2016). Attachment of the blastocyst to the uterine luminal epithelium occurs at a point equidistant between openings of the uterine glands. The polar trophoblast overlying the inner cell mass adheres preferentially, presumably reflecting the different properties of this subpopulation. Malrotation of the blastocyst at this stage will lead to the connecting stalk, the forerunner of the umbilical cord, not being centralized over the developing placenta, resulting in an eccentric, or even a velamentous cord insertion. Heparin-binding epidermal growth factor–

like growth factor (HB-EGF) expressed by the uterine luminal epithelium has been identified as a key factor in the initial attachment, binding to members of the EGF family of receptors present on the surface of the trophoblast (Cha et al., 2012). Later, integrins, L-selectin ligands and selectin oligosaccharides are also involved.

Our knowledge of events during, and immediately following implantation is extremely limited due to the inaccessibility of the tissues. Attempts have been made to mimic the process *in vitro*, culturing blastocysts on monolayers of endometrial cells (Lindenberg et al., 1989, Bentin-Ley et al., 2000). These have confirmed that implantation is of the intrusive type, with protrusions of trophoblast penetrating between and displacing the uterine cells. Where the trophoblast cells make contact with the epithelium they undergo proliferation and differentiation, fusing with neighbors to form small areas of multinucleated syncytial trophoblast. The same phenomenon occurs when blastocysts are cultured on plastic, suggesting it does not require biological signals from another tissue (Shahbazi et al., 2016). Mitotic figures are not seen within the syncytial masses, and the deeper trophoblast cells that formed the original wall of the blastocyst remain uninucleate and represent a progenitor population, the cytotrophoblast cells. Some of these cells most likely represent true trophoblast stem cells, but as yet molecular markers that characterize such cells unequivocally have yet to be identified.

The most extensive collection of human implantation material is the Carnegie Collection. The earliest specimen available (Carnegie stage 5a) shows a partially embedded conceptus; the uterine epithelium has been penetrated but not yet grown over the surface of the sac (Fig. 1). A flattened mass of syncytial trophoblast is apposed to the endometrium and performs the initial invasion (Enders, 1989), and hence this is referred to as the trophoblastic plate stage of development. Nuclei within the syncytial masses are of different sizes and chromatin appearances, possibly reflecting either fusion between the trophoblast and maternal

cells, or a progression towards polyploidy (Enders, 2001). The mural trophoblast on the opposite pole of the gestational sac remains unicellular and projects into the uterine lumen. There is little evidence of transformation of the endometrial stromal cells into decidua.

Although human implantation is of the interstitial type and described as invasive this terminology may create a false impression, for there is evidence that upgrowth of the endometrial cells plays an important role in encapsulating the conceptus. Thus, the original descriptions of human early implantation sites describe mitotic figures in the endometrial stromal cells close to the conceptus (Hertig and Rock, 1941). More recent experimental data also support the concept of trophoblast-induced proliferation and subsequent migration around, and over, the conceptus to form the decidua capsularis (Gellersen et al., 2010). This view is compatible with high-resolution ultrasound scans showing the implantation site raised above the general level of the endometrial surface, bulging into the uterine lumen. The conceptus therefore always lies in the superficial layer of the endometrium, and it is regression of the underlying endometrium, by then converted into the decidua basalis, that allows it to sink deeper into the uterine wall (Hempstock et al., 2004).

The lacunar stage of placental development

With the re-establishment of the uterine epithelium at stage 5b, (~day 8 p-f), the syncytial trophoblast expands to cover the entire chorionic sac (Fig. 2). A key step in placental development occurs at the same time when fluid-filled spaces, referred to as lacunae, appear in the syncytial mass. As these enlarge and coalesce they subdivide the mass into two layers, one in contact with the endometrium, the forerunner of the basal plate, and one in contact with the cytotrophoblast cells of the original blastocyst wall, the forerunner of the chorionic plate. This separation establishes the boundaries of the placenta, with a series of pillars of syncytial trophoblast, the trabeculae, extending between the two and representing forerunners of the

anchoring villi.

During this process, a new type of syncytial trophoblast arises that resembles the syncytiotrophoblast of the definitive placenta. It forms a unilaminar microvillous polarized epithelium that lines the lacunae, and represents a transition from the early invasive form to one suited for absorption and transport processes (Enders, 1989).

At its outer surface, the original syncytial trophoblast erodes into the maternal capillaries within the superficial endometrium, which are dilated in the vicinity of the implantation site. Consequently, erythrocytes are released into the lacunae, although they are surprisingly few in number and have pale-staining characteristics (Hertig et al., 1956, Hamilton and Boyd, 1960). Movement, if any, through the lacunae must be like a slow venous ebb and flow (Hamilton and Boyd, 1960), and does not represent an effective maternal arterial circulation. The early syncytial trophoblast also surrounds neighboring uterine glands (Hertig and Rock, 1941, Enders, 1989). The gland epithelial cells appear degenerate at these sites, establishing communications with the lacunae and enabling the continuance of histotrophic nutrition despite the interstitial form of implantation.

During this stage of development, cells derived from the hypoblast layer of the embryonic germ disc grow round the inner surface of the cytotrophophoblast cells, forming the primary yolk sac. Shortly after, extraembryonic mesoderm differentiates between the two tissues. The origin of the extraembryonic mesoderm in the human is still uncertain. Early reports suggested that it arises by delamination from the inner surface of the trophoblast (Hertig and Rock, 1941), but the fact that the trophoblast rests on a well-defined basement membrane (Knoth and Larsen, 1972) renders this unlikely. An alternative suggestion was that the mesoderm results from gastrulation that occurs at the most caudal end of the primitive streak before the main streak is established, the cells migrating over the inner surface of the trophoblast rather than penetrating the germ disc (Luckett, 1978). Studies of macaques

indicated an origin from the hypoblast layer as a further possibility (Enders and King, 1988). Recent support for this observation comes from molecular analyses demonstrating that the two tissues share a number of common markers, in particular high expression of *GATA4* and *GATA6* (Nakamura et al., 2016). Although the precise origin remains uncertain, present evidence indicates that the extraembryonic mesoderm arises from the hypoblast layer or jointly from the epiblast and hypoblast layers (Boss et al., 2018). Once differentiated, extraembryonic mesoderm rapidly extends over the inner surface of the trophoblast. Henceforth, the two layers are referred to as the chorion, and the blastocyst as the chorionic sac.

Around day 12 p-f, the cytotrophoblast cells lying beneath the syncytiotrophoblast proliferate and penetrate into the trabeculae as cellular columns. Approximately two days later the cells reach the tips of the trabeculae, whereupon they extend through the syncytiotrophoblast and spread laterally, coalescing with neighbours to form a new layer interposed between the syncytiotrophoblast and the endometrium, the cytotrophoblastic shell (Boyd and Hamilton, 1970, Burton and Jauniaux, 2017). The importance of the shell will be considered later.

Early villus development

Continued cytotrophoblast cell proliferation results in side branches forming on the trabeculae and protruding into the lacunae. These branches represent primary villi, and are composed of a cytotrophoblast cell core with a covering of syncytiotrophoblast. Their presence marks the beginning of the villous stages of placentation. Further proliferative activity, with branching of the primary villi, initiates the development of primitive villous trees. As the trees are derived from the former trabeculae, they are always continuous with the developing chorionic

plate. At the same time, the lacunar system is, by definition, transformed into the intervillous space.

After a further two days, around day 15 p-f, mesenchymal cells derived from the extraembryonic mesoderm lining the developing chorionic plate invade the primary villi, transforming them into secondary villi. Within a few days, the mesenchyme extends towards the villous tips, but never reaches as far as the cytotrophoblastic shell. Rather, the distal segments of what are now referred to as anchoring villi remain as cytotrophoblast cell columns. These columns are largely devoid of a covering of syncytiotrophoblast. A stem/progenitor cell niche persists at the proximal end of each column adjacent to the mesenchymal core where the cytotrophoblast cells continue to proliferate (Hemberger et al., 2010, Lee et al., 2018). Daughter cells differentiate under the influence of Notch signaling (Haider et al., 2016), and lose their mitotic potential as they move distally along the column. Ultimately they feed into the shell. The length of the columns reduces as pregnancy advances, reflecting the decline in proliferative potential of the cytotrophoblast cells after the end of the first trimester (Hemberger et al., 2010, Soncin et al., 2018).

Beginning between days 18 and 20 p-f, the first fetal capillaries can be observed within the mesenchyme of the secondary villi and the developing chorionic plate. They form *in situ* from hemangioblastic progenitor cell clusters, which in turn differentiate from the mesenchyme (Dempsey, 1972, Demir et al., 1989, Robin et al., 2009, Aplin et al., 2015). The same progenitor cells give rise to groups of hematopoietic stem cells that form nucleated erythrocytes surrounded by the developing endothelium. The appearance of capillaries in the villous mesenchymal stroma marks the development of the first tertiary villi.

Initially, villi form over the complete surface of the chorionic sac creating the chorion frondosum (Fig. 3), and by the end of this period the essential framework of the placenta is established.

The cytotrophoblastic shell

The cytotrophoblastic shell is best developed in early pregnancy when it is several cells thick and provides a means for rapid circumferential expansion of the implantation site (Hamilton and Boyd, 1960). The cells have a distinctive phenotype, being rounded and containing large amounts of glycogen (Figs 3 and 4). The shell helps create a unique microenvironment in which placental development is stimulated, while at the same time protecting the embryo against the potentially teratogenic effects of reactive oxygen species during the critical phase of organogenesis (Jauniaux et al., 2003a). In particular, when the outer surface of the shell encounters the tip of a maternal spiral artery, cells migrate down the lumen of the artery as endovascular trophoblast. The volume of this migration is such that the trophoblast effectively 'plugs' the mouths of the spiral arteries, restricting any inflow into the placenta to a slow seepage of plasma and largely excluding erythrocytes (Fig. 4d) (Hamilton and Boyd, 1960, Hustin et al., 1988, Burton et al., 1999, Saghian et al., 2019). Towards the end of the first trimester, channels develop progressively within the plugs (Burton et al., 1999, Roberts et al., 2017), but contrast-enhanced ultrasound has revealed that flow does not significantly increase until around 12-13 weeks of gestation. Thus, it is thought that more proximal segments of the utero-placental vasculature may be rate limiting, in particular the radial arteries that do not undergo remodeling until the end of the first trimester (Roberts et al., 2017). This view is supported by computational models of the utero-placental vasculature that reveal the radial arteries to be a site of major resistance within the network (Clark et al., 2018). Overall, the effect is that placental development from implantation until the end of the first trimester occurs in a physiological low-oxygen environment of approximately 20 mmHg (Jauniaux et al., 2000, Jauniaux et al., 2001).

This environment should not be considered hypoxic as has sometimes been the case, for there is no evidence that the tissues are energetically compromised (Cindrova-Davies et al., 2015, Gorr, 2017). Instead, phylogenetically old carbohydrate metabolic pathways involving polyols are highly active, allowing glycolysis to continue at a high rate without reliance on fermentation to lactate (Jauniaux et al., 2005, Burton et al., 2017). These pathways are closely linked to the pentose phosphate pathway, and have the added advantage that carbon skeletons are preserved and can be used for synthesis of nucleotides in support of cell proliferation. The one requirement is a rich supply of glucose, and this is provided by the histotroph from the uterine glands, as will be discussed later. Polyols are also powerful osmolytes and may facilitate the drawing of water across the placenta to expand the coelomic and amniotic cavities.

Plugging of the spiral arteries during early pregnancy appears essential, and has also been observed in the rhesus macaque (Ramsey and Donner, 1980). Poor development of the shell is associated with early onset of maternal blood into extensive areas of the placenta and spontaneous miscarriage, irrespective of the embryonic karyotype (Hustin et al., 1990, Jauniaux et al., 2003b). High levels of oxidative stress are observed in the placental tissues along with widespread degeneration of the syncytiotrophoblast (Hempstock et al., 2003b). Mechanical disruption of the maternal-fetal interface through intrauterine subchorionic hematomas may also contribute lead to focal oxidative stress and weakening of the placental membranes (Johns et al., 2006, , Burton and Jauniaux, 2017).

Placental development during the first trimester

Once the main framework of the placenta is established, there is a period of rapid growth, far in excess of that of the embryo. New villi are formed from the lateral aspects of the anchoring villi as a result of villous sprouts that arise from the syncytiotrophoblast (Burton and Jones,

2009). Initially, these club-shaped projections contain only syncytioplasm, but may contain aggregates of euchromatic nuclei in the expanded head. The proximal end is invaded by first cytotrophoblast and then by mesenchymal cells so that they undergo the same primary, secondary and tertiary stages seen following implantation. The original anchoring villi form the stem villi of the definitive placenta, and the new villi form progressively smaller branches, generations of intermediate villi that are free floating. Terminal villi start to differentiate only after 20 weeks of gestation (Jackson et al., 1992), and so are not seen at this stage.

Villi during the first trimester have a low surface area to volume ratio, are relatively poorly vascularized, and so not well adapted to facilitate exchange (Fig. 4c). The trophoblast epithelium is two layered, with an outer layer of syncytiotrophoblast and a complete underlying layer of cytotrophoblast cells. The majority of these cells immunostain positively for CDX2, a marker of trophoblast stemness, and for Ki67, a proliferation marker, suggesting they are undergoing active division (Burton et al., 2020). This prolific expansion may be facilitated by the low oxygen concentration, which maintains embryonic stem cells in a pluripotent state (Lees et al., 2017), and stimulates trophoblast proliferation in first trimester primary cultures (Genbacev et al., 1996) and explant models (Caniggia et al., 2000).

The mesenchymal cells of the villous core are quite sparse, and have long sail-like processes that unite with neighbors to form a meshwork enclosing fluid-filled channels (Martinoli et al., 1984, Burton, 1987). At the proximal end of the stem villi these channels appear to be in free communication with the coelomic cavity of the chorionic sac, and hence may facilitate diffusion of oxygen and nutrients into the cavity in the absence of an effective chorionic circulation. Villous macrophages, also referred to eponymously as Hofbauer cells, are frequently observed within the channels (Fig. 4c) (Castellucci et al., 1980). They are thought to arise initially by differentiation *in situ* from the hemangioblastic cultures (Demir et al., 1989), but once the feto-chorionic circulation is established at around 8-10 weeks of

pregnancy they may be supplemented by bone-marrow derived monocytes. Semi-quantitative estimates indicate they represent approximately 40% of all stromal cells, both in early pregnancy and at term (Goldstein et al., 1988). They can be easily recognized by their large size, 10-35 μm in diameter, and rounded appearance with a highly vacuolated cytoplasm. It is thought they perform immune surveillance (Reyes et al., 2017), but may also have an important in regulating placental morphogenesis. Later in pregnancy they are closely approximated to vascular structures and the trophoblast basement membrane, and are immunopositive for a number of growth factors (Benirschke et al., 2012).

During the first trimester the endometrial stromal cells undergo the decidual transformation. While this change may occur during the secretory phase of the non-pregnant cycle, it is very limited in its extent. In the early weeks of a pregnancy the transformation becomes more extensive, involving the endometrium around the entire uterine wall. The region beneath the developing placenta is henceforth referred to as the decidua basalis, and the remainder of the uterine lining as the decidua parietalis. The thin layer overlying the implantation site is the decidua capsularis. Decidual cells are characterized by their enlarged size and rounded shape, and secrete a number of factors, including prolactin, IGF-binding protein, and relaxin. The decidua plays an important role in modulating trophoblast invasion as evidenced by the deep, unregulated invasion seen at sites of ectopic implantation, such as in the Fallopian tube, where decidua is absent. The decidua is also essential for supplying histotrophic nutrition to the conceptus during early pregnancy.

Histotrophic nutrition

The communications between the uterine glands and the placenta seen at the time of implantation persist throughout the first trimester (Hamilton and Boyd, 1960, Burton et al., 2002, Moser et al., 2015), enabling an extended period of histotrophic nutrition prior to onset

of the maternal placental circulation. The epithelial cells lining the glands undergo a characteristic morphological change during early pregnancy, referred to as the Arias-Stella reaction (Arias-Stella, 2002). They adopt a hypersecretory phenotype that appears to be endocrine mediated as it occurs even in ectopic pregnancies. Glycogen accumulates within the apical cytoplasm of the cells, and may be released through a combination of apocrine secretion and conversion into glucose through the actions of glycogen phosphorylase (Demir et al., 2002, Jones et al., 2015). Lipid droplets are also prominent in the secretions (Hempstock et al., 2004). The secretions are delivered into the intervillous space through openings in the cytotrophoblastic shell, and later the developing basal plate (Burton, 2002 #157}(Hempstock et al., 2004). They disperse within the intervillous space and immunofluorescence studies confirm uptake by the syncytiotrophoblast where they co-localise with the lysosomal pathway (Hempstock et al., 2004). Breakdown of maternal proteins may provide the amino acids and elements required for anabolic pathways within the trophoblast similar that seen in the rodent yolk sac (Brent and Fawcett, 1998), but some, such as glycodelin-A, pass across the placenta intact and accumulate within the coelomic (chorionic) and amniotic cavities (Seppälä et al., 1992, Jauniaux and Gulbis, 2000).

The glands are also an important source of growth factors, including epidermal growth factor (EGF), vascular endothelial growth factor and leukemia inhibitory factor (Hempstock et al., 2004). Application of EGF to first trimester villous explants stimulates proliferation of the cytotrophoblast cells (Maruo et al., 1992), and hence it has been proposed that the histotroph plays an important role in stimulating placental proliferation and differentiation as in domestic species (Filant and Spencer, 2014, Burton, 2018, Burton et al., 2020). Obtaining proof of an equivalent servo-mechanism by which hormones from the placenta upregulate expression of growth factors within the glands is difficult in the human, but the presence of the Arias-Stella reaction provides support. In addition, the recent demonstration that early

pregnancy hormones, such as human chorionic gonadotropin and prolactin, upregulate secretion of glycodelin-A and osteopontin from uterine gland organoids is strongly indicative (Turco et al., 2017). Prolactin is not secreted by the trophoblast in the human as in other species (Carter, 2012), but by the decidua. Correct decidualisation and subsequent paracrine signaling to the glands may therefore be essential for placental development and a successful pregnancy (Conrad et al., 2017).

The amnion

The amnion arises from the epiblast layer of the inner cell mass (Fig. 3). The cells form a cluster, or rosette, polarize and then a central cavity emerges (Deglincerti et al., 2016, Shahbazi et al., 2016), in a similar fashion to that observed in the rhesus monkey (Enders et al., 1986). The cells of the epiblast remain columnar, while those of the amnion are more squamous. The sac gradually expands as gestation advances, extending on to the ventral surface of the embryo following folding of the latter, and sheathing the connecting stalk to form the umbilical cord. The amnion finally makes contact with the chorionic plate at the end of the 3rd month, obliterating the coelom.

Formation and role of the yolk sac

The primary yolk sac is formed by hypoblast cells growing around the inner surface of the trophoblast, but later undergoes extensive remodeling when the layer of extraembryonic mesoderm interposed between the two vacuolates and splits into two layers. The outer layer lines the trophoblast and contributes to the chorion as described earlier, and the inner layer covers the yolk sac, forming its outer mesothelial layer. The space between the two layers is the coelomic cavity. At the same time, the yolk sac reduces in size as the more peripheral portions are nipped off, forming the secondary yolk sac. This sac reaches a maximum

diameter of 6-7 mm between the 6th and 10th weeks of gestation, after which it decreases.

The secondary yolk sac is connected to the gut tube of the embryo by the vitelline duct, and is surrounded by the coelomic fluid. Morphologically it consists of two epithelial layers, an outer mesothelial layer and an inner endodermal layer, separated by a small amount of mesenchyme in which the vitelline vessels differentiate. The mesothelial layer is formed of flattened cells that display all the features of an absorptive epithelium, with a dense covering of microvilli, coated pits and pinocytotic vesicles (Gonzalez-Crussi and Roth, 1976, Jones and Jauniaux, 1995). By contrast, the endodermal cells are more columnar and contain large quantities of rough endoplasmic reticulum, Golgi bodies and secretory droplets, indicative of a synthetic function. It is notable that the vitelline capillary plexus lies closely approximated to mesothelial epithelium.

The secondary human yolk sac fails to makes contact with the chorion (Fig. 5), and a choriovitelline placenta is never formed as in other species. Hence, the yolk sac has largely been considered vestigial, although it is recognized as an important early site of hematopoiesis in the embryo (Pereda and Niimi, 2008). It is also known to synthesise several key serum proteins, including alpha-fetoprotein, alpha$_1$-antitrypsin, albumin and transferrin, prior to the fetal liver having differentiated sufficiently to take on this role (Buffe et al., 1993, Jones and Jauniaux, 1995). Recently, the potential importance of the yolk sac for transfer of nutrients has been highlighted by RNAseq data that confirm the presence of transcripts encoding a broad array of transporter proteins for lipids, amino acids, metal ions and other micronutrients (Cindrova-Davies et al., 2017). The conservation of the majority of these transcripts across the mouse and chicken where there is strong experimental evidence of the role of their encoded proteins in transport indicates that the human yolk sac may be more active than previously appreciated. In addition, immunohistochemistry confirms the presence of transporter proteins, such as tocopherol transfer protein, GLUT1 and transferrin, which are

primarily localized to the outer mesothelial layer (Jauniaux et al., 2004, Benirschke et al., 2012). The presence of the ligands for many of the transporters in the coelomic fluid bathing the epithelium adds further support for a potential role of the yolk sac in maternal-fetal transport (Cindrova-Davies et al., 2017).

It has been proposed, therefore, that the human yolk sac contributes to a physiological choriovitelline placenta during early pregnancy (Fig. 5), with the coelomic cavity interposed between the trophoblast and the yolk sac acting as an intermediary nutrient reservoir (Cindrova-Davies et al., 2017). From the evidence available it would appear to be most important for the handling and metabolism of cholesterol and lipids, which are essential as signaling intermediates and for formation of cell and organelle membranes (Woollett, 2008). By the 9th week of pregnancy the yolk sac begins to show morphological evidence of a decline in function, and when the coelomic cavity is obliterated at around 15 weeks the remnants of the yolk sac become incorporated into the umbilical cord. Abnormal development of the yolk sac has been linked with miscarriage (Nogales et al., 1993, Freyer and Renfree, 2009), but whether this is cause or effect is unknown at present.

The extravillous trophoblast

As the name implies, extravillous trophoblast (EVT) extends beyond the confines of the villous trees, invading into the decidua where it plays an essential role in remodeling of the maternal spiral arteries. The EVT originates from the cytotrophoblastic cell columns and the cytotrophoblastic shell as previously described. Cells on the outer surface of the shell undergo an epithelial-mesenchymal transition (EMT), changing shape from rounded profiles to pleiomorphic spindle-shaped cells that stain darkly with hematoxylin and eosin and express placenta-specific protein 8, PLAC8, that promotes invasiveness (Fig. 4c) (Chang et al., 2018). The stimulus for the transition is not known, but cells in the distal part of the column are

immunopositive for CDCP1, which represses epithelial and promotes mesenchymal states (Wong et al., 2019). The transcription factor ZEB2 has recently been shown to potentially play a key role in regulating the transition, for overexpression in BeWo and JEG3 choriocarcinoma cell lines stimulates a transcript profile indicative of an EMT and increases cell invasiveness (DaSilva-Arnold et al., 2019). Whether expression of ZEB2 is responsive to the increased concentration of oxygen in the decidua or factors secreted by the decidual cells is not known at present. Measurements of DNA suggest that invasive extravillous trophoblast cells undergo endoreduplication and are tetraploid (Zybina et al., 2002, Zybina et al., 2004, Velicky et al., 2018), and at the same time start to express markers of senescence.

Extravillous trophoblast cells migrate via two routes; the endovascular down the lumen of the arteries and the interstitial through the endometrial stroma where they cluster around the spiral arteries and the endometrial glands. Invasion of the interstitial trophoblast is extensive, and in a normal pregnancy they reach the inner third of the myometrium where they fuse with neighbours to form multinucleated placental bed giant cells (Al-Lamki et al., 1999). No active function has been attributed to these cells, although they are immunopositive for human placental lactogen, subunits of hCG (Al-Lamki et al., 1999), and members of the angiopoietin and vascular endothelial growth factor families (Schiessl et al., 2009). Potentially, they may promote regeneration of the endometrium following delivery.

The regulation of the invasion is complex, and numerous factors have been shown to influence invasion positively and negatively *in vitro* (Benirschke et al., 2012). In reality, it will depend on the local concentration of cytokines, glycoproteins, and possibly oxygen, within the decidua (Lee et al., 2011, Pollheimer et al., 2018). An important source of cytokines are cells of the maternal immune system. There is strong genetic evidence that HLA-C ligands on the surface of the interstitial cells bind with killer-like immunoglobulin receptors (KIR) on the maternal uterine Natural Killer (uNK) cells, causing activation of the

latter (Hiby et al., 2010). Despite the terminology, there is no evidence of killing of the trophoblast, but rather the converse. Cytokines released from the uNKs, in particular GM-CSF, attract trophoblast cells (Abbas et al., 2017), and are thought to play an important role in remodeling of the spiral arteries (Moffett et al., 2015).

Interstitial trophoblast cells express a unique hyperglycosylated form of human chorionic gonadotropin (hCG-H), which functions as a cytokine rather than a hormone (Cole, 2010, Evans, 2016). hCG-H is a powerful stimulus for trophoblast invasion, acting in an autocrine fashion through the TGF-ß receptor rather than the LH receptor as for normal hCG. As might be expected, concentrations in maternal serum are highest during early pregnancy, and are significantly reduced in cases that go on to early pregnancy loss (Cole, 2007, Guibourdenche et al., 2010). Monitoring hCG-H may therefore provide an assay of extravillous trophoblast numbers, if not invasion.

Remodeling of the spiral arteries

Connecting the placenta to the maternal arterial system poses a hemodynamic challenge due to the high pressure and velocity within the latter. The spiral arteries that ultimately supply the placenta must therefore undergo considerable remodeling to minimise the risks of damage to the delicate villous trees and mechanical disruption of the maternal-fetal interface. Remodeling, also referred to as physiological conversion or transformation, involves the loss of smooth muscle and the elastic lamina from the walls of the vessels, and its replacement with an inert fibrinoid material (Fig. 4d). These changes normally involve the endometrial portion of the artery and the portion within the inner third of the myometrium.

Remodeling starts with an endocrine priming that is associated with decidualisation and affects all spiral arteries, even those in the decidua parietalis on the opposite wall of the uterus. The endothelial cells swell and there is a loosening of the smooth muscle cells

(Pijnenborg et al., 2006, Whitley and Cartwright, 2010, Harris, 2010). More extensive changes require the presence of extravillous trophoblast, and involve the de-differentiation of the vascular smooth muscle cells and their migration away from the vessel wall. Apoptosis of the smooth muscle cells has been suggested, but it is not thought to play a major role (Bulmer et al., 2012). Initially, the endothelial cells are also lost and replaced by endovascular trophoblast, but later the vessels are re-endothelialised by maternal cells that grow along the vessels and can extend onto the inner surface of the placental basal plate (Ockleford, 2010).

Remodeling has two important consequences for placental blood flow. Firstly, it is associated with dilation of the terminal parts of the vessels as they approach the basal plate (Harris and Ramsey, 1966). Mathematical modelling reveals that the dilation reduces the velocity of the inflowing blood by an order of magnitude to ~10 cm/s, allowing sufficient transit time for effective maternal-fetal exchange. At the same time, it reduces the pressure in the intervillous space and preventing compression and collapse of the fetal capillary network within the villi (Burton et al., 2009). Secondly, by inactivating the hypercontractile segment of a spiral artery that lies in the junctional zone just beneath the endometrial-myometrial boundary, remodeling ensures a constant flow of blood irrespective of maternal vasomotor events. The hypercontractile segment normally contracts at the time of menstruation to prevent excessive blood loss, but contraction during pregnancy could impair the maternal-fetal supply line and lead to fluctuations in the intraplacental oxygen concentration. Experiments *in vitro* have demonstrated that hypoxia-reoxygenation is a powerful inducer of oxidative stress in placental tissues (Hung and Burton, 2006), more so than hypoxia alone, and can recapitulate many of the changes seen in pre-eclampsia (Cindrova-Davies et al., 2007).

Failure of remodeling is associated with the most common complications of human pregnancy, including growth restriction, pre-eclampsia and pre-term delivery (Brosens et al.,

2011). The effects are mediated by a combination of reduced villous development and surface area for exchange, oxidative stress and release of pro-inflammatory factors from the placenta (Kingdom et al., 2018, Burton and Jauniaux, 2018, Burton et al., 2019).

The formation of the definitive placenta

Starting at ~8th week of pregnancy, the villi over the superficial pole of the chorionic sac begin to appear shorter and less vascularized compared to those over the deep pole in contact with the decidua basalis (Fig. 6) (Hamilton and Boyd, 1960). This is a progressive effect, so that eventually only 'ghosts' of villi comprising an avascular and almost acellular villous core with a thin covering of trophoblast, remain in this area (Fig. 7). In the past this regression of the villi to form the chorion laeve or smooth membranes has been attributed to a lack of blood supply reaching them from the decidua capsularis. However, it is notable that the villi are often surrounded by masses of maternal erythrocytes, and an alternative hypothesis has been put forward relating to the onset of the maternal arterial circulation. In normal pregnancies, onset of the circulations starts preferentially in the peripheral parts of the placenta where the endovascular aggregates in the spiral arteries are least extensive (Jauniaux et al., 2003b). Villi sampled from this region display high levels of oxidative stress and activation of the apoptotic cascade compared to their central counterparts (Jauniaux et al., 2003b, Burton et al., 2010). Thus, it has been proposed that the high local levels of oxygen induce villous regression through oxidative stress and not a lack of maternal circulation.

There are pathological parallels to this pattern of regression, for in cases of spontaneous miscarriage onset of the maternal circulation is both precocious and disorganized, occurring in both the peripheral and central regions (Jauniaux et al., 2003b). As mentioned previously, there is widespread oxidative damage to the trophoblast and other tissues, and if the placenta remains in the uterus for a period of time the villi become

avascular collagenous ghosts that closely resemble those of the chorion laeve (Jauniaux et al., 2003b, Hempstock et al., 2003b). These findings support the concept that under both physiological and pathological conditions, elevated levels of oxygen cause villous regression.

Whatever the mechanism, it appears that the shape of the placenta and the centrality or otherwise of the cord insertion seen at term is determined by events taking place at the end of the first trimester (Fig 7b) (Schwartz et al., 2011, Salafia et al., 2012).

Growth of the definitive placenta

Once established, the definitive placenta continues to expand over the second and third trimesters. New stem and intermediate villi are generated through the transformation of syncytial sprouts as mentioned earlier, and slowly increase in volume across gestation (Jackson et al., 1992). By contrast, elaboration of the functional units of the placenta, the terminal villi, increases exponentially from ~20 weeks until term (Fig. 8a) (Jackson et al., 1992). This elaboration ultimately provides a villous surface area of 12-14 m^2, with a diffusion distance of 7-8 μm separating the maternal and fetal circulations (Burton and Jauniaux, 1995). The theoretical diffusing capacity of the placenta can be calculated from these parameters, and if expressed per kg of fetal weight the value stays remarkably constant across gestation (Mayhew et al., 1993). This finding suggests a close relationship between the development of the fetus and the structural parameters of the placenta.

The chorionic and basal plates become better defined as gestation advances. The vessels in the chorionic plate distributing umbilical blood flow to the stem villi increase in caliber (Fig. 8a). The decidua basalis continues to thin, and the remaining decidual cells become enmeshed in a fibrinoid matrix with the remnants of the cytotrophoblastic shell to form the basal plate (Fig. 8b). Infoldings of the basal plate form septa that sub-divide the placenta into a series of lobes; each lobe may contain one of more lobules as below. The septa

do not extend as far as the chorionic plate, but nonetheless may serve to direct and partially compartmentalise maternal blood flow (Ramsey and Donner, 1980).

Despite remodeling of the spiral arteries, the maternal blood still enters the placenta in jet-like spurts that gradually diminish in velocity as gestation advances (Collins et al., 2012). The momentum of the blood is sufficient to sculpt the villous trees into a series of lobules, each with a central villus-free cavity located over the opening of an artery. Once delivered into that cavity, the maternal blood disperses through the clefts or pores between neighboring villi, before draining into the uterine veins. Each lobule therefore acts as an independent maternal-fetal exchange unit, physiologically equivalent to a cotyledon of a ruminant placenta. Measurements of the expression and activity of antioxidant enzymes indicate that an oxygen gradient exists across a lobule, with an arterial centre and more venous periphery (Hempstock et al., 2003a). This pattern of blood flow and oxygenation has recently been confirmed using magnetic resonance imaging techniques (Hutter et al., 2020).

The elaboration of the villous trees is accompanied by an increase in the volume of the trophoblast, both syncytiotrophoblast and cytotrophoblast. Although markers of stemness in trophoblast progenitors decline at the end of the first trimester, proliferation and fusion continues, possibly involving a transit amplifying population or equivalent. Consequently, the number of nuclei in both subtypes increases until term (Simpson et al., 1992). However, as villous surface area enlarges rapidly, the cytotrophoblast layer becomes discontinuous, and cells are only occasionally observed in any one section, creating a false impression of their total number. This discontinuity contributes to the reduction in villous membrane thickness and facilitates diffusional exchange.

The majority (~80%) of the nuclei within the syncytiotrophoblast are transcriptionally active, as evidenced by incorporation of fluorouracil and immunopositivity for phosphorylated upstream binding factor (pUBF) and phosphorylated cAMP response element

binding protein (pCREB) that are indicative of RNA polymerase I (RNA Pol I) and RNA pol II driven transcription respectively (Ellery et al., 2009, Fogarty et al., 2011). The proportion of active nuclei remains constant across gestation.

Some of the active nuclei are also immunopositive for proliferating cell nuclear antigen (PCNA), indicating that they have only recently entered the syncytiotrophoblast; PCNA has a half-life of more than 20 h (Fogarty et al., 2011). The chromatin pattern of the syncytial nuclei varies greatly, with those that are transcriptionally inactive generally showing greater heterochromatin. Aggregates of nuclei with extremely condensed chromatin, referred to as syncytial knots (Burton and Jones, 2009), are seen increasingly towards term, and in the past it has been suggested that these represent apoptotic changes (Huppertz et al., 1999, Huppertz and Kaufmann, 1999). However, the majority of the nuclei are negative for TUNEL staining (Coleman et al., 2013), and it is now accepted that apoptosis does not occur in the normal syncytiotrophoblast (Longtine et al., 2012b, Longtine et al., 2012a). Instead, it is thought that the chromatin condensation reflects epigenetic changes, for the nuclei in syncytial knots stain strongly for 5'hydroxymethylcytosine (Fogarty et al., 2015). They are also immunopositive 8-oxo-deoxyguanosine, indicative of oxidative damage (Fogarty et al., 2013), and so the following life cycle has been proposed. Nuclei entering the syncytiotrophoblast are transcriptionally active, but a proportion gradually acquire oxidative damage and are shut-down epigenetically. Whether this is a purely temporal phenomenon or responsive to the local microenvironment within the intervillous space is not known. The effete nuclei are aggregated into syncytial knots at sites on the villous surface where they do not impinge on placental functional capacity, but the mechanism of aggregation is not known (Coleman et al., 2013, Calvert et al., 2016).

As the villi mature their stromal core becomes more condensed, and the channels seen during the first trimester are gradually obliterated (Fig 8c). The fetal vasculature becomes

more prominent, particularly in terminal villi where the capillaries may represent ~35% of the villous volume. The capillaries follow a tortuous course, forming a complex network with many interconnections (Jirkovska et al., 2002, Plitman Mayo et al., 2016a). The diameter of the capillaries varies along their length, with narrow sections interspersed with dilated regions, referred to as sinusoids, usually located on the point of an acute bend (Plitman Mayo et al., 2019). The dilation brings the outer wall of the capillary into close contact with the trophoblast basement membrane and the syncytiotrophoblast, which is thinned as a consequence of the distending pressure of the capillary. These areas are referred to as vasculosyncytial membranes, and the thickness of the villous membrane may be reduced to as little as 2 μm. Computational modeling reveals that they are the principal sites of diffusional exchange (Plitman Mayo et al., 2016b). Hence, the condition of terminal villus deficiency, when terminal villi fail to form, is associated with growth restriction and fetal hypoxaemia (Khong et al., 2016).

With the enlargement of the chorionic sac, the decidua capsularis becomes apposed to the decidua parietalis on the opposite wall of the uterus (Fig. 7) at approximately weeks 10-12, and fuses. By now the villi have completely regressed over the chorion laeve, but the extravillous trophoblast of the original cytotrophoblastic shell in this region form a layer 5-10 cells thick (Fig. 9a). This layer stains immunopositively for HLA-G and cytokeratin and is apposed to the decidual cells, but no invasion takes place (Fig. 9b).

Placental senescence

The concept of placental ageing and senescence is an old one (Martin and Spicer, 1973, Rosso, 1976), but has received renewed interest following the finding that cell fusion to form a syncytium induces senescence changes (Chuprin et al., 2013, Cox and Redman, 2017). Senescence can also be induced by chronic oxidative stress, and as mentioned previously

oxidatively damaged nuclei accumulate in the syncytiotrophoblast towards term in the form of syncytial knots (Fogarty et al., 2013). The damage is thought to arise from an increasing mismatch between maternal supply and feto-placental demand leading to fluctuations in the oxygen concentration within the intervillous space. Markers of senescence are variable in the normal term placenta, but oxidised DNA and lipids and the expression p21, p16 and cGAMP are all greatly increased in the post-mature placenta and in pathological cases (Maiti et al., 2017, Cindrova-Davies et al., 2018). Senescence and impairment of placental functions may therefore contribute to the increased risk of stillbirth and neonatal death in post-mature pregnancies.

Comparison with other primates

A comparison of placentation in the human with that across the order Primates is beyond the scope of this chapter, and detailed accounts can be found elsewhere (Mossman, 1987, Wooding and Burton, 2008, Carter and Pijnenborg, 2011). Here we focus on two species of Old-World monkeys that have been extensively used for placental and reproductive research, the rhesus macaque and the baboon, and the great apes. Macroscopically, the structure and development of the placental villous trees are very similar across all these, but there are differences in the mode of implantation and invasion of the extravillous trophoblast, and the complexity of the immunological interactions.

Implantation in the macaque and baboon is superficial, and so no decidua capsularis forms and the blastocyst remains within the uterine lumen exposed to the endometrial secretions. Whether this influences the route for histotrophic nutrition has not been addressed. In the baboon only one placental disc is formed at the site of implantation (Houston, 1969), whereas in the macaque a bi-discoidal placenta is common, with a second placental disc developing 11-12 days later where the blastocyst impinges on the opposite uterine wall

(Enders, 2007, de Rijk and van Esch, 2008). There is thus no equivalent of the regression of villi of the chorion frondosum to form the definitive placenta as in the great apes and human.

Nonetheless, where the trophectoderm contacts the uterine epithelium there is transformation into a primary syncytiotrophoblast that is initially invasive, and a progenitor population of cytotrophoblast cells. A thick cytotrophoblastic shell is formed in both the macaque and the baboon, and unlike in the human it has a sharp demarcation with the endometrium and persists throughout pregnancy (Enders et al., 2001). Endovascular trophoblast invasion is prolific in both, and aggregates of endovascular cells plug the spiral arteries in the macaque as in the human, restricting flow of maternal arterial blood into the placenta during early pregnancy (Ramsey and Donner, 1980, Blankenship and Enders, 2003). By contrast with the human, interstitial trophoblast is extremely limited, and there are no multinucleated giant cells in the placental bed (Pijnenborg et al., 1996). This calls into question the role of the interstitial trophoblast in the human, and it may be that they are more important for paracrine signaling to stimulate secretions from the endometrial glands than for arterial remodeling (Burton et al., 2020). Another significant difference is that remodeling of the spiral arteries is restricted to only the endometrial portion, and does not extend into the myometrial segment in the baboon (Pijnenborg et al., 1996). In the chimpanzee and gorilla trophoblast invasion occurs via the endovascular and interstitial routes, and extends as far as the inner third of the myometrium, as in the human (Pijnenborg et al., 2011a, Pijnenborg et al., 2011b).

These differences in extravillous trophoblast invasion are associated with marked co-evolution of the KIR and HLA-C systems. Uterine NK cells are present in the placental bed of the rhesus macaque but HLA-C is absent on the trophoblast cells. An HLA-C1 epitope evolved in the orangutan from an *MHC-B* ancestor, and a C2 epitope is found in the gorilla, chimpanzee and human. The highest degree of complexity is seen in the human where the

KIR A and *B* haplotypes have evolved (Parham and Moffett, 2013, Moffett and Colucci, 2015). Certain combinations of the *KIR* and *HLA-C* haplotypes predispose to complications of human pregnancy, depending on the degree of inhibition and activation of the KIR. Thus the interactions between KIR-A/A and C2/C2 are predominantly inhibitory and associated with an increased risk of miscarriage, growth restriction and pre-eclampsia (Hiby et al., 2010). Indeed, these immune interactions appear to regulate birth weight across the entire range, from growth restriction to macrosomia (Moffett et al., 2015), presumably acting through the degree of remodelling of the spiral arteries and the effect on utero-placental blood flow.

Conclusions

Placentation in the human and closely related great apes displays major morphological differences compared to other orders of mammals, in particular the degree of invasiveness of the trophoblast. Physiologically, there are greater similarities; the reliance on histotrophic nutrition during the period of organogenesis, the importance of the yolk sac during early pregnancy, and the large surface area and thin interhaemal membrane to facilitate exchange. Human pregnancy is associated with a high rate of pregnancy complications, such as pre-eclampsia, and genetic analyses reveal this predisposition may be powerful driver of placental evolution towards a less invasive state (Elliot and Crespi, 2015).

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Figure legends

Figure 1. Human blastocyst implanting at Carnegie stage 5A, approximately seven days p-f. a) The trophoblast in contact with the endometrium has differentiated into a primary syncytiotrophoblast (syntr) that contains nuclei of different sizes and chromatin patterns, and a population of underlying uninucleate cytotrophoblast progenitor cells. The mural trophectoderm of the abembryonic pole of the blastocyst is draped over the inner cell mass (ICM) and is still exposed to the uterine lumen as the uterine epithelium (ue) has not yet reformed. eg, endometrial gland. b) A section towards the margin of the implantation site showing the primary syncytiotrophoblast (syntr) eroding into the wall of an endometrial gland (eg), making the histotroph available. Photomicrograph courtesy of Prof. AC Enders and the Carnegie Collection.

Figure 2. Photomicrograph of a human conceptus at Carnegie stage 5B. The uterine epithelium (ue) has almost completely regrown over the implantation site. Fluid-filled spaces, the lacunae (l) have developed in the syncytiotrophoblast (syntr), and contain occasional erythrocytes released from an endometrial capillary eroded by the trophoblast (arrow). The syncytiotrophoblast also appears to be eroding into an endometrial gland (eg). The inner cell mass has differentiated into an epiblast layer that is continuous with the amnion, enclosing the amniotic cavity (ac), and hypoblast layer that grows around the inside of the cytotrophoblast cells (asterisk) to form the primary yolk sac (pys). Photomicrograph courtesy of Prof. AC Enders and the Carnegie Collection.

Figure 3. Photomicrograph of a human conceptus at Carnegie stage 7, approximately 15-17 days p-f. The decidua capsularis (dc) now covers the conceptus which lies in the superficial endometrium. Villi (v) cover the entire surface of the chorionic sac. The villi are at the

secondary stage, having a covering of trophoblast and a core of extra-embryonic mesenchyme (eem). They are separated by the lacunae (l). At their tips the villi are continued as a cytotrophoblast cell column (cc) of extravillous trophoblast cells, and these merge with neighbours at the maternal-fetal interface to create the cytotrophoblast shell (cs). By comparison, the embryo within the coelomic cavity (c) is relatively poorly developed, comprising a columnar epiblast layer that is continuous with the amnion (a) and the hypoblast that is continuous with the primary yolk sac (pys). Photomicrograph courtesy of Prof. AC Enders and the Carnegie Collection.

Figure 4. Photomicrographs of a developing placenta at approximately four weeks p-f. a) The chorionic sac is covered by the decidua capsularis (dc) and projects into the uterine lumen above the general line of the endometrium (e) which is asterisked. The chorionic sac has been opened on the superficial aspect and the embryo removed. Villi can be seen arising from the developing chorionic plate (cp). There has been haemorrhage into some of the endometrial glands. m, myometrium. b) The mesenchymal stromal core does not extend to the tips of the anchoring villi (av) which terminate as the cytotrophoblast cell columns (ccc). Cytotrophoblast cells proliferate at the proximal ends of the columns, differentiate into extravillous trophoblast as they move down the column, and merge with neighbors to form the cytotrophoblastic shell (cs) at the maternal-fetal interface. At the maternal surface of the shell, cells undergo an epithelial-mesenchymal transition to form darkly staining spindle shaped interstitial trophoblast (arrowed) that invade into the endometrium (e). c) The epithelial covering of the villi comprises an outer multinucleated layer of syncytiotrophoblast (s) and an underlying layer of uninucleate villous cytotrophoblast progenitor cells (c). The mesenchymal cells of the core are loosely packed and sail-like processes form fluid-filled stromal channels (sc) in which villous macrophages (arrowed) are often observed. d)

Specimen at nine weeks p-f showing a spiral artery outlined by deposition of pink–staining fibrinoid plugged by endovascular trophoblast (et). Ink injected into the uterine artery can be seen within the intercellular spaces reaching the cytotrophoblastic shell (cs), indicating the possibility of a slow seepage of plasma into the intervillous space. Scale bars; 5 mm, 250 µm, 100µm, and 100 µm respectively. Stain a-c Masson's trichrome, d hematoxylin and eosin.

Figure 5. Diagrammatic comparison of the nutrient pathway during early pregnancy in the mouse (A), and the speculated pathway in the human (B). In the mouse, histotroph from the endometrial glands (EG) is phagocytosed (1) by the endodermal cells (E) of the visceral layer of the inverted yolk sac (YS). Following fusion with lysosomes (2), digestion of maternal proteins leads to release of amino acids that are transported (3) to the fetal circulation (FC). In the human, histotroph released into the intervillous space (IVS) is phagocytosed (1) by the syncytiotrophoblast (STB). Following digestion by lysosomal enzymes (2), free amino acids may be transported (3) by efflux transporters to the coelomic fluid (CF) where they accumulate. Nutrients in the CF may be taken up by the mesothelial cells (M) of the yolk sac and transported (4) into the fetal circulation (FC). Alternatively, they may diffuse into the cavity of the yolk sac and be taken up by the endodermal cells (5). Some intact maternal proteins may also be released into the CF by exocytosis of residual bodies (6), and be engulfed by the mesothelial cells (7). CTB; cytotrophoblast cells. Reproduced with permission from (Cindrova-Davies et al., 2017).

Figure 6. Photomicrograph of a specimen at six weeks p-f. The chorionic sac covered by the decidua capsularis (dc) projects into the uterine lumen, and has been opened to remove the embryo. The decidua capsularis has not yet fused with the decidua parietalis (dp) lining the rest of the uterine wall. Villi (asterisk) towards the superficial pole of the chorionic sac are

less extensive than those over the deep pole in contact with the decidua basalis (db). The interface with the decidua basalis is very uneven at this stage. Glands within the decidua are still active. cp, chorionic plate. Scale bar, 10 mm. Stain, Masson's trichrome.

Figure 7. Photographs of placenta-in-situ at a) seven weeks, and b) 12 weeks p-f. a) Villi covering the superficial pole of the chorionic sac are now less extensive and avascular compared to those over the deep pole. The decidua capsularis has not yet fused with the decidua parietalis and a small part of the uterine cavity (uc) remains. b) By now the villi have fully regressed, creating the chorion laeve or smooth membranes (arrowed) that are draped over the decidua parietalis (dp) of the uterine wall opposite. The extent of the definitive placental disc is marked by the asterisks. The meshwork of placental villi within the disc is finer and denser than previously, and the decidua basalis (db) is thinner and more even. A small portion of the uterine cavity (uc) persists. Stain, hematoxylin and eosin.

Figure 8. Photographs of a) and b) placenta-in-situ at ~30.5 weeks p-f, and c) placental villi at term. a) The density of the villus trees continues to increase with the elaboration of intermediate and terminal villi. Infoldings of the basal plate create septae that partially separate the placenta into a series of lobes. The chorion laeve (arrowed) is apposed to the decidua parietalis. b) Higher power photomicrograph of the basal plate (bp), which comprises a complex mix of extravillous trophoblast arising from the original cytotrophoblastic shell and decidual cells enmeshed in fibrinoid material. Under the basal plate is a loose meshwork of tissue that acts as a plane of cleavage at the time of delivery. c) Photomicrograph of terminal villi from a term placenta embedded in resin and sectioned at 1 μm. The fetal capillaries display localized dilations that bring the endothelium close to the synctiotrophoblast to form a vasculo-syncytial membrane (arrowed). The distance between

the maternal and fetal circulation is reduced to ~2 µm at these sites, which facilitate diffusional exchange. A cytotrophoblast cell undergoing mitosis is asterisked. Scale bars; b) 200 µm, c) 20 µm. Stains; a) and b) hematoxylin and eosin, c) methylene blue.

Figure 9. Photomicrograph of placental membranes at a) 20 weeks p-f, and b) at term. a) The membranes comprise the amniotic epithelium (arrowed), extraembryonic mesenchyme (eem), and a layer of extravillous trophoblast (ct) that abut the cells of the decidua parietalis (dp). Endometrial glands (eg) are still evident in the decidua parietalis at this stage. b) Immunostaining against cytokeratin confirms the presence of the cytotrophoblast cells (ct) that abut the decidua parietalis which now only contains rudimentary endometrial glands (eg). Scale bars, 250 µm. Stain; a) hematoxylin and eosin; b) anti-pancytokertain.

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