Chapter 8

Placental function in intrauterine growth restriction

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Introduction

Appropriate fetal growth in utero depends on a variety of factors including:

- paternally- and maternally-derived fetal genetic factors;
- maternal nutritional and hormonal factors;
- uterine environment, including the placenta.

An imbalance between paternal and maternal genetic factors, suboptimal nutritional supply from the mother to the fetus, and a dysregulation of placental development may all cause intrauterine growth restriction (IUGR). During pregnancy, the placenta is the decisive organ between mother and fetus and brings the blood systems of both individuals in close vicinity to one another to insure appropriate nutrient and oxygen supply to the fetus.

Features of growth restriction

IUGR affects approximately 5% of all pregnancies and is the second leading cause of perinatal mortality and morbidity [1,2]. As mentioned in previous chapters, there is often confusion in defining appropriate fetal growth, leading to the terms small for gestational age (SGA) and IUGR being used synonymously. However, SGA is a 'soft term' that includes all newborns with a birth weight below the tenth percentile. A number of these babies have used their appropriate growth potential

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and are genetically small, but otherwise normal [3]. By contrast, IUGR is a pathological subgroup within SGA: for all infants born SGA with a birth weight below the tenth percentile for gestational age, only 30% can also be classified as IUGR [4]. A clear delineation between SGA and IUGR was achieved by extending the definition of IUGR to include infants with a birth weight below the tenth percentile, as well as an abdominal circumference below the tenth percentile, or a longitudinal decrease in the growth of the abdominal circumference of more than 40 percentiles independently from the age-specific size curve [5].

Additionally, typical features of IUGR, such as alterations of blood flow in the uterine and umbilical arteries are now also used to classify IUGR [5,6]. Blood flow alterations within the maternal uterine arteries have been attributed to an inadequate invasion and transformation of the downstream spiral arteries [5]. Hence, partial vasomotor control of these vessels by the mother remains and results in pulsatile flow of maternal blood towards the placenta [7]. Even more disadvantageous for fetal growth and overall well-being are alterations of blood flow within the umbilical arteries. Such changes may result in an increased systole/diastole ratio in cases with still preserved end diastolic flow. Further alterations may lead to the absence of end diastolic flow velocity in these arteries or end diastolic flow may even be reversed [6]. The causes of such alterations of umbilical arterial blood flow include problems such as malformations of the villous tree, resulting in increased peripheral resistance of placental vessels.

Early trophoblast development

During human embryonic development the trophoblast lineage develops as the first cell lineage. First, trophoblast cells appear at the blastocyst stage with the trophectoderm covering the inner cell mass and the blastocyst cavity. During implantation, further differentiation of the trophoblast into the mononucleated cytotrophoblast and the multinucleated syncytiotrophoblast is crucial for the invasion of the early embryo into uterine tissues. Both trophoblast subpopulations further develop into various subtypes (Figure 8.1), establishing all trophoblast populations necessary for proper placental and fetal development. The two major

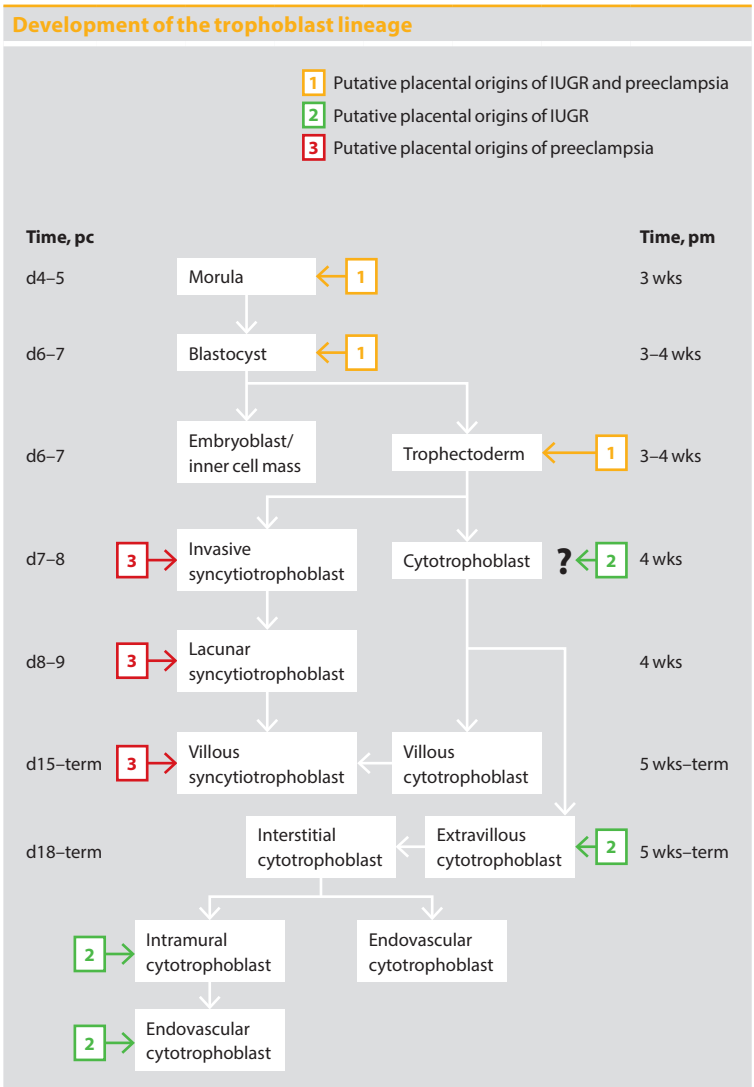

Figure 8.1 Development of the trophoblast lineage. The time of appearance can be found with day post-conception on the left and week post-menstruation (pm) on the right. The numbers in colored boxes indicate the putative insults or dysregulation of trophoblast development causing IUGR and/or preeclampsia: 1, Very early defects will affect all trophoblast subtypes and cause IUGR and preeclampsia; 2, Defects of the extravillous trophoblast in certain subtypes of this population will cause idiopathic IUGR. Defects in the cytotrophoblast, rather than only the extravillous trophoblast, may cause idiopathic IUGR; 3, Defects in the development of the syncytiotrophoblast at various stages will cause preeclampsia. IUGR, intrauterine growth restriction.

subpopulations of the trophoblast during pregnancy are the villous trophoblast and the extravillous trophoblast. The villous trophoblast is the epithelial cover of all placental villi and constitutes the placental barrier. The extravillous trophoblast is found outside the placental villi, mostly in the placental bed. Here this subpopulation invades the uterine wall and transforms the spiral arteries according to the needs of the growing fetus.

Extravillous trophoblast invasion

During the early stages of placental development, trophoblastic cell columns develop at the tips of anchoring villi (Figure 8.2). Here the subset of trophoblasts in direct contact to the villous basement membrane proliferates and is the source of all extravillous trophoblasts invading into maternal tissues. As soon as the trophoblasts lose contact with the basement membrane, they also lose their proliferative capacity and differentiate towards an invasive phenotype. From the cell columns, the extravillous trophoblasts invade into the decidual stroma; this is why they are termed 'interstitial trophoblasts' (Figure 8.2). Interstitial trophoblast is the initial subset of extravillous trophoblast with the potential to differentiate into further subtypes all of which are important for appropriate fetal growth. Interstitial trophoblasts may remain interstitial and invade maternal tissues down to the inner third of the myometrium, invade uterine glands and become endoglandular trophoblast, or invade spiral arteries. In the latter case, they first differentiate into intramural trophoblasts in the vessel walls and then further differentiate into endovascular trophoblasts at the inner surface or in the lumen of the arteries. The pathway of choice may be chosen at different depths of invasion as depicted in Figure 8.2.

Transformation of spiral arteries by extravillous trophoblast invasion

Three stages of spiral artery transformation have been identified that subsequently enable adequate supply of the placenta and fetus:

Stage 1: Vascular changes within the uterine wall independent of trophoblast invasion. Maternal vessels within the uterine walls are initially

modified by the mother as soon as she becomes pregnant. Such modifications comprise widespread perturbations of the spiral arteries, vacuolation, and basophilia of the endothelium, disorganization of the vascular smooth muscle cells, and dilation of the vessel lumen [8]. These vessel modifications occur throughout the whole uterus and are not directly linked to trophoblast invasion [8].

Stage 2: Remodeling of spiral arteries by interstitial trophoblasts in close vicinity to the vessel wall. This step has been described in the guinea pig and is anticipated to occur in the human as well [9–11]. Those interstitial trophoblasts that come into close vicinity to spiral arteries secrete factors such as nitric oxide to further remodel the vessel wall and to widen the lumen. Additionally, these secreted factors further reduce the number of smooth muscle cells in the vessel wall, leading to the deposition of fibrinoid in the media prior to infiltration by endomural trophoblasts. Hence, this step comprises changes of cell numbers and extracellular matrix composition in the vessel walls [12].

Stage 3: Infiltration of the vessel wall and establishment of the endovascular trophoblast. After priming the walls of the spiral arteries, intramural trophoblasts infiltrate the vessel wall and further reduce the number of smooth muscle cells and elastic fibers [13,14]. The lumen of the spiral arteries now becomes dilated, reaching several times the original diameter of the untransformed spiral artery [15–17]. The intramural trophoblasts finally reach the endothelial basement membrane, pass this layer, and replace the endothelial cover of the vessels. These vessels are now lined by endovascular trophoblasts that start to crawl along the endothelial lining to further replace this layer. By means of deep interstitial trophoblast invasion and subsequent infiltration of vessels, trophoblasts transform spiral arteries down to the inner third of the myometrium.

Invasion of intramural and endovascular trophoblasts is a crucial step to convert maternal spiral arteries into large-bore conduits that mediate the adequate supply of oxygen and nutrients to the placenta and thus the fetus [17,18]. This supply of oxygen and nutrients is only established at the end of the first trimester [19]. During the first 10–12 weeks of gestation, endovascular trophoblasts not only replace the endothelial

Schematic representation of extravillous trophoblast invasion into maternal tissues at the beginning of the second trimester of pregnancy

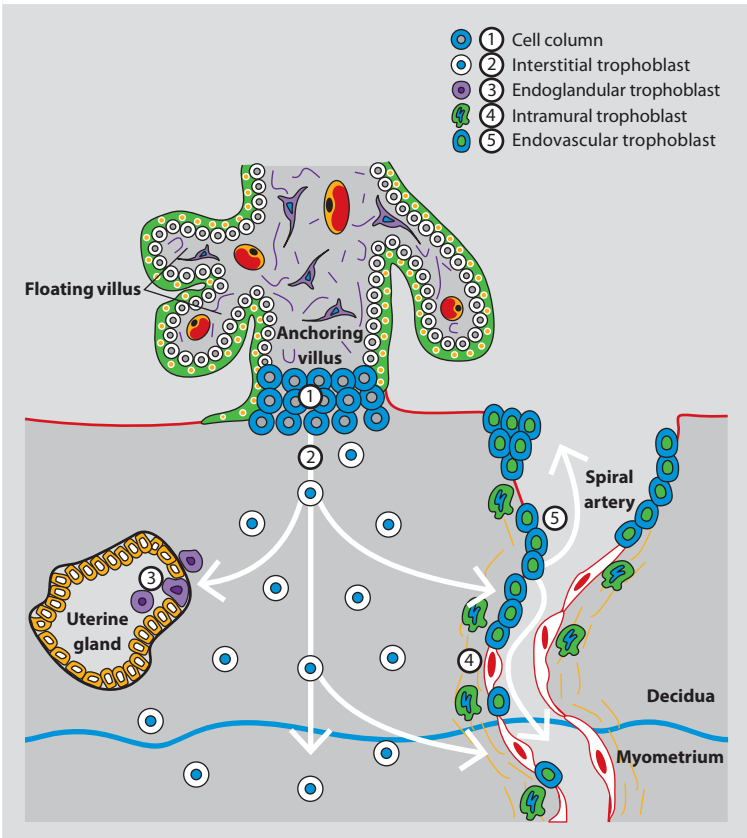

Figure 8.2 Schematic representation of extravillous trophoblast invasion into maternal tissues at the beginning of the second trimester of pregnancy. 1, Anchoring villi are attached to the decidua by trophoblast cell columns, which are the source of all extravillous trophoblasts; 2, As soon as invasive interstitial trophoblasts detach from the cell columns, they start invade into the decidual stroma, finally reaching the inner third of the myometrium. Alternative routes of invasion originating from the interstitial trophoblast go towards uterine glands; 3, endoglandular trophoblast, or towards spiral arteries; 4, intramural trophoblast; 5, endovascular trophoblast.

lining of spiral arteries but generate large aggregates of cells that plug up the vessel lumen. By this means, no maternal blood cells can enter the intervillous space and the placental villi are submerged by a lake of plasma, ultrafiltrated by the trophoblast aggregates in the lumen of the spiral arteries. Adequate nutrition of the embryo during the first

trimester of pregnancy is supplied by plasma and secretion products of the uterine glands (histiotrophic nutrition), which are eroded by endoglandular trophoblasts [20] and opened towards the intervillous space [19,21]. After 10–12 weeks of gestation, the plugs of endovascular trophoblasts become permeable and only now can maternal blood cells enter the intervillous space and establish the maternal blood flow to the placenta (hemotrophic nutrition).

Thus, during the first half of pregnancy, uterine spiral arteries within the placental bed go through a series of pregnancy-specific modifications comprising:

- replacement of smooth muscle cells in the vessel media by endomural trophoblast and loss of vasomotor control;
- degradation of elastic fibers and loss of elasticity;
- widening into dilated, incontractile tubes; and
- replacement of endothelial cells by the endovascular trophoblast [22].

Transformation of spiral arteries results in a dramatic decrease in the velocity of blood flow towards the intervillous space from 1–2 m/s to approximately 10 cm/s and only has a modest impact on total blood volume flowing into the placenta [7]. At the same time, loss of maternal vasomotor control, as well as loss of contractility, assures sufficient blood supply from the mother to the placenta at any time [10,17]. Transformation of maternal uterine spiral arteries into uteroplacental vessels is vital for normal fetal growth and development.

Intrauterine growth restriction and alterations of trophoblast and placenta

Due to the close correlation between abnormal uterine artery Doppler waveforms and development of IUGR, there is general agreement that IUGR is directly linked to impaired trophoblast invasion and subsequent failure of transformation of spiral arteries.

Alterations of the extravillous trophoblast

The link between inadequate trophoblast invasion and IUGR with preeclampsia was first reported in 1972 [23]. Since then, trophoblast

invasion has remained one of the major foci in placental research. Today, it is generally accepted that in cases with IUGR, it is mostly the invasion of the spiral arteries rather than the general interstitial invasion that is affected. In cases with IUGR, the interstitial trophoblast is reduced in number, but apoptosis is not increased. By contrast, the intramural and endovascular trophoblast are not only reduced in number but also show significantly increased rates of apoptosis [24]. Reduction of both trophoblast subtypes can explain why the respective vessels show a constricted lumen compared to normally invaded vessels. Moreover, maternal macrophages in close vicinity to intramural trophoblasts may further decrease the number of trophoblasts by secretion of tumor necrosis factor-alpha and indolamine-2,3-dioxygenase, a tryptophan degrading enzyme [25].

Besides this impaired invasion into the decidua, deep invasion into the inner third of the myometrium is reduced in cases with IUGR, especially into the walls of spiral arteries in this area. Here, a highly contractile segment of the spiral arteries can be found, which is inactivated during normal invasion. In IUGR, this segment is still active, causing spontaneous vasoconstrictions and intermittent, rather than uninterrupted, perfusion of the placenta [7]. It has to be stressed that the aforementioned observations have been seen in the placental bed after delivery [7]. We can only speculate on the causes and pathways that lead to these changes, but clear observations on how these alterations developed are not yet available.

Effects of alterations of trophoblast invasion

In a normal pregnancy, only the final endings of the spiral arteries are widened, while the deeper parts of the uterine arterial system remain unchanged. The major effect of this widening is to reduce the velocity of blood flow into the placenta by a factor of 100–200, to velocities of about 10 cm/s. Under such conditions, maternal blood enters the intervillous space uninterrupted and with a laminar flow [7].

Interestingly, impaired invasion of spiral arteries by extravillous trophoblasts in IUGR only has a modest impact on the blood volume flowing into the placenta [7]. Accordingly, the availability of nutrients and oxygen in

the intervillous space should not be different compared to normal blood flow. Hence, placental hypoxia cannot be deduced from such a flow pattern [26]. At the same time, problems in fetoplacental circulation can still cause fetal hypoxia without any signs of placental hypoxia.

Impaired invasion of the uteroplacental arteries causes a dramatic increase in the velocity of maternal blood flowing into the intervillous space, reaching a speed of 1–2 m/s [7]. In this scenario, maternal blood enters the intervillous space with high speed and a turbulent flow pattern. This change in flow velocity has dramatic consequences for the villous trees.

Damage of the villous architecture

The epithelial cover of the floating villi (villous syncytiotrophoblast) is a very fragile layer and may be damaged by the high velocity of blood in direct contact with this layer. This damage can be visualized after delivery by a thickening of the villous basement membrane, increased deposition of fibrin-type fibrinoid, and villous infarction [27].

Rupture of anchoring villi

In the presence of an increased velocity of maternal blood flowing into the placenta, it is thought that anchoring villi break off from the decidua and the respective trophoblast cell columns disintegrate [9]. Destruction of the cell columns subsequently results in a reduction in the pool of extravillous trophoblasts and may explain the reduced number of interstitial trophoblasts at delivery.

Increased peripheral resistance in placental vessels

The increased velocity of maternal blood flow into the placenta also leads to a partial increase in pressure in the intervillous space, which will have an impact on the fragile placental villi; their capillary system cannot withstand the increased pressure and thus will reduce in width. This causes increased peripheral resistance in the placental vasculature, which may have an adverse impact on the fetal vascular system [5,6]. This may result in reduced flow in the umbilical arteries, which is a common feature associated with IUGR.

Alterations of the villous trophoblast in intrauterine growth restriction

On the level of the villous cytotrophoblast, IUGR cases show significant differences compared to age-matched controls in terms of total cytotrophoblast volume, total number of cytotrophoblasts, and total number of Ki-67 positive cytotrophoblasts as a measure of cytotrophoblast proliferation [28]. Hence, the villous cytotrophoblast shows obvious alterations in cases of IUGR.

In cases of IUGR, the lower number of villous cytotrophoblasts directly affects the syncytiotrophoblast by reducing its volume and total number of nuclei. Interestingly, the aforementioned alterations are not present in cases of pure preeclampsia [28]. Such defects in villous trophoblast growth may have an impact on the transport of nutrients from maternal to fetal blood. Also, the increased velocity of maternal blood passing the placental villi may reduce the ability of the trophoblast to take up a sufficient amount of nutrients to guarantee an appropriate feeding of the fetus.

It still needs to be clarified if the alterations found in the villous trophoblast are direct effects of idiopathic IUGR and represent a defect in the development of the trophoblast lineage. The alternative explanation would favor a secondary impact due to the alterations of blood flow in the intervillous space as described above. The second scenario would place an impact on the development of only the extravillous trophoblast and a subsequent impact on the villous trophoblast due to changes in blood flow.

Alterations of the placenta in intrauterine growth restriction

Placentae from healthy controls and placentae from patients with pure preeclampsia do not differ in regards to total placental volume and total volume of all placental villi [29,30]. Also, the volumes of specific types of villi (eg, stem, intermediate, and terminal) do not show any significant differences. By contrast, in idiopathic IUGR cases, all of the above values are significantly reduced. The placentae are smaller, there are less and smaller villi, and there is a trend towards more fibrinoid deposition in the placenta, indicating more damage to the tissues [28–30].

Placental origins of intrauterine growth restriction and preeclampsia

Dysregulation at various stages of development of the trophoblast and its subtypes, may result in an inadequate differentiation of the respective subtype (Figure 8.1). Dysregulation during the early establishment of the trophoblast lineage will have an impact on all subpopulations of the trophoblast, especially villous and extravillous trophoblasts (Figure 8.1). Alterations may already occur prior to blastocyst formation (ie, on the level of sperm and/or egg, zygote, or the first blastomeres prior to development of the morula), or at the time of blastocyst formation. Dysregulation of trophoblast differentiation may also occur slightly later when the first cytotrophoblasts are formed, which subsequently develop into villous and extravillous cytotrophoblasts. In all of these scenarios, any major dysregulation of trophoblast development will have an impact on placental development as a whole. Accordingly, the result may be a combination of preeclampsia and IUGR and could explain the severe early onset cases of patients suffering from both preeclampsia and IUGR.

Dysregulation of the extravillous pathway of trophoblast development may lead to idiopathic IUGR (Figure 8.1). In this scenario, trophoblast invasion is inadequate and transformation of spiral arteries may not be sufficient. This alteration may only occur on the level of the subtype of endomural/endovascular trophoblast, while the other subtypes of extravillous trophoblast may not show major alterations. Hence, the typical features of IUGR – failure of trophoblast invasion and missing transformation of the uterine arteries – could be explained with this scenario. In Figure 8.1, one defect causing IUGR is labeled with a question mark. This is to illustrate that it is not clear yet whether there is general defect of the cytotrophoblast subpopulation in IUGR or whether only the extravillous type of trophoblast is affected.

Dysregulation of the villous pathway of trophoblast development may lead to preeclampsia (Figure 8.1). Dysregulation of villous syncytiotrophoblast during early stages of gestation may result in malfunction and abnormal turnover, resulting in the discharge of nonapoptotic (ie, necrotic or aponecrotic) trophoblastic particles that are already affecting the mother at this stage of pregnancy [31,32]. Quantification

of the preeclampsia-specific biomarker placental protein 13 (PP13) has revealed that alterations in serum PP13 can be detected at 7 weeks gestation [33]. In this scenario, only the villous trophoblast is affected, resulting in the initiation of an inflammatory response in the mother and thus, the clinical symptoms of preeclampsia. The extravillous pathway of trophoblast development is not affected in preeclampsia, since only a small subset of preeclampsia cases (ie, less than 20%) are further affected by IUGR and inadequate trophoblast invasion [33].

Conclusion

IUGR remains a major cause of fetal mortality and morbidity during pregnancy. It appears that dysregulation in the development of the extravillous trophoblast can explain most of the placental alterations that are typical for idiopathic IUGR. At the same time, it has become clear that IUGR and preeclampsia are indeed different entities that may occur at the same time, placing an even stronger burden on mother and baby. A thorough analysis and comparison of both syndromes is mandatory to decipher the different etiologies of preeclampsia and IUGR.

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