The Placenta

Basics and Clinical Significance

Berthold Huppertz
Ekkehard Schleußner
Editors

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Foreword

Dear Reader,

Why a textbook on the placenta?

Every person has this organ for only 9 months, after which it is disposed of or, at best, a tree is planted on it. And yet it is one of the most exciting subjects imaginable! Within a very short time, the placenta has to develop, take up its function, make a successful pregnancy possible, supply and protect the unborn child—and at the same time influence all bodily functions of the pregnant woman in such a way that the resources are also available for all the above-mentioned processes.

When Springer-Verlag asked whether I, Berthold Huppertz, would like to publish a textbook on the placenta, the publishing staff did not know that this was and is a long-cherished wish of me as an enthusiastic placentologist.

Now there is the challenge of publishing a textbook on the placenta that would meet my requirements as a basic scientist, but at the same time be appealing to clinically active colleagues. It was therefore clear at a very early stage that an obstetrician had to be found as a partner. This was quickly found in Ekkehard Schleußner—with his "favourite organ, the placenta."

Together we defined topics, found friends and colleagues as co-authors, and finally we dared to tackle this project.

There are many textbooks in the fields of pregnancy and obstetrics, and some of them have small sections on the placenta. Some decades ago, there was also a very good atlas of placental morphology by Martin Vogel—also published by Springer. But until today (until this book!), there is no textbook on the human placenta in German language, which covers and combines both morphology and function, physiology and pathology, as well as basic research and clinic. With this English version, this textbook is now also available for a larger audience.

Anyone who has followed the publications on the subject of placenta in recent years will have noticed that the last decade alone has been marked by a large number of new findings that have led to fundamental changes in the understanding of pregnancy complications. However, these are often known to only a small community and rarely find their way into clinical research and even more rarely into clinical practice. This is where we see the great opportunity for our book. It is clear that a textbook will always be too slow to publish all these innovations directly. However, a textbook can summarize the latest insights and relate them to each other. Moreover, it can present many disciplines together in an attempt to link them together. Examples include morphology and ultrasound or function and predictive biomarkers.

Our hope now is that we have succeeded in putting together a textbook that meets many demands—and from different fields and areas. We are sure that you as an

obstetrician and midwife, as a pathologist or basic researcher will each find important suggestions and new insights for your work.

As editors, we would like to thank all the co-authors for their commitment and the publisher for his continuous support. We are pleased to have realized our dream and hope you enjoy reading it.

Yours,

Berthold Huppertz
Graz, Austria

Ekkehard Schleußner
Jena, Germany
December 2017

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Berthold Huppertz
has been studying the human placenta, especially the trophoblast, and its alterations in IUGR and preeclampsia for more than two decades.

Ekkehard Schleußner
is an obstetrician working on placental insufficiency and its clinical consequences. The focus of the placental laboratory of the Department of Obstetrics is placental physiology in the placental perfusion model and immunology at the maternoplacental interface.

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Abbreviations

11β-HSD	11-beta-hydroxysteroid dehydrogenase
ACTH	Adrenocorticotrophic hormone
AEDF	Absent End-Diastolic Flow
AIP	Abnormally Invasive Placenta
APLS	Antiphospholipid Syndrome
cAMP	Cyclic adenosine monophosphate
CBD	Cannabidiol
CE	Chronic endometritis
CHD	Chorionic cavity diameter
CMV	Cytomegalovirus
CRH	Corticotropin Releasing Hormone
CRL	Crown-rump length
CTG	Cardiotocography
DC	Dichorial
DHEA	Dehydroepiandrosterone sulfate
DIC	Disseminated intravascular coagulopathy
DOHaD	Developmental Origins of Health and Disease
DWI	Diffusion weighting
EFW	Estimated Fetal Weight
EVT	Extravillous trophoblast
FACS	Fluorescent Activated Cell Sorting
FGR	Fetal Growth Restriction
FOAD	Fetal Origins of Adult Disease
FSH	Follicle Stimulating Hormone
GDM	Gestational diabetes
GHRF	Growth Hormone Releasing Factor
GLUT	Glucose transporter
GnRH	Gonadotropin Releasing Hormone
GR	Glucocorticoid Receptor
HbA	Adult hemoglobin
HBC	Hofbauer cell
HbF	Fetal hemoglobin
hCG	Human Chorionic Gonadotropin
HELLP Syndrome	Hemolysis, Elevated Liver enzymes, Low Platelets (syndrome)
HLA	Histocompatibility antigen
HPA	Hypothalamus-Pituitary-Adrenal (-axis)
HUAEC	Human Umbilical Artery Endothelial Cell
HUVEC	Human Umbilical Vein Endothelial Cell
IDO	Indoleamine-2,3-dioxygenase
IFN-γ	Interferon gamma
IGF	Insulin-like Growth Factor
IL	Interleukin
IUFD	Intrauterine Fetal Death
IUGR	Intrauterine Growth Restriction
IVF	In Vitro Fertilization
LBW	Low Birth Weight
LH	Luteinizing Hormone
MACS	Magnetic Cell Sorting
MC	Monochorial
MCDA	Monochorial diamniotic
MCMA	Monochorial monoamniotic
MDMA	3,4-methylenedioxymethamphetamine
METH	Methamphetamine

MPS	Massive Parallel Sequencing
MVM	Microvillous Membrane
NGS	Next Generation Sequencing
NICHD	National Institute of Child Health and Human Development
NIPT	Non-Invasive Prenatal Testing
NK cells	Natural Killer cells
NOS	Nitric Oxide Synthetase
oGTT	Oral Glucose Tolerance Test
p.c.	post conception
PAPP-A	Pregnancy-Associated Plasma Protein A
PCOS	Polycystic Ovary Syndrome
PED	Preserved End-Diastolic flow
PGE2	Prostaglandin E2
PGF/PlGF	Placental Growth Factor
PIBF	Progesterone-Induced Blocking Factor
PID	Preimplantation Genetic Diagnosis
PL	Placental Lactogen
PRL	Prolactin
REDF	Reversed End-Diastolic Flow
RSA	Recurrent Spontaneous Abortions
SGA	Small for Gestation Age
SLE	Systemic Lupus Erythematosus
SNP	Single-nucleotide polymorphisms
SWI	Susceptibility weighting
THC	Tetrahydrocannabinol
TNF-α	Tumor Necrosis Factor alpha
Treg	Regulatory T cell
TSH	Thyroid-Stimulating Hormone
TTTS	Twin-to-Twin Transfusion Syndrome
VEGF	Vascular Endothelial Growth Factor

Placental Development with Histological Aspects

Berthold Huppertz

Contents

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1.1 Introduction

The placenta was already recognized and revered by the early Egyptians. It was then the Greek physician Diogenes of Apollonia in the 5th century BC who was the first to describe the function of the organ in nourishing the fetus. Aristotle (384–322 BC) then followed with the description that the fetal membranes completely enclose the fetus. It was not until the Renaissance in 1559 that Matteo Realdo Colombo introduced the term "placenta", which is traced back to the Latin root for a flat cake.

1.2 Development of the Placenta

1.2.1 Prelacunar Phase (Days 5 to 8 after fertilization)

On the 5th day after fertilization (day 5 p. c., post conception), the first cell line of the embryo, the trophoblast, has differentiated. This has led to the formation of the blastocyst. The trophoblast cells form as a spherical envelope around the embryoblast even before implantation, forming the outer envelope of the blastocyst, the trophectoderm. In the blastocyst cavity, which is enclosed by the trophectoderm, the inner cell mass of the blastocyst, the embryoblast, is found on one side in direct contact with the cells of the trophectoderm.

The trophoblast cells subsequently appear exclusively in the placenta, while the cells of the embryoblast are not only responsible for the entire structure of the embryo, but also provide other cells for the placenta (villous stroma and chorion). The umbilical cord and the amnion also arise from cells of the embryoblast.

The blastocyst, which adheres to the uterine epithelium at implantation (day 6-7 p. c.) and subsequently invades the connective tissue of the endometrium, consists of 107–256 cells. The majority of these cells are trophoblast cells in the trophectoderm. The most common site of implantation in the uterus is found in the upper portion of the posterior wall of the uterus, near the midsagittal axis.

The symmetry of the blastocyst (top-bottom, right-left) is determined by the position of the embryo in the blastocyst and leads, among other things, to the attachment of only those trophoblast cells to the uterine epithelium that are in direct contact with the embryo. Abnormal orientation of the blastocyst with attachment of trophoblast cells that are not in contact with the embryo may cause abnormalities in umbilical cord insertion. These abnormal orientations appear to be more common in pregnancies from in vitro fertilization (IVF) (Baergen 2011).

During implantation, the trophoblast of the blastocyst proliferates to such an extent that it becomes multilayered. During this process, the outer trophoblast cells, which adhere to the uterine epithelium and are the first to penetrate this epithelium, fuse, giving rise to the first syncytiotrophoblast, which is invasive at this stage. This initially oligonucleated, later multinucleated syncytium is formed by the fusion of individual trophoblast cells, first with each other, later only by fusion of individual cells with the syncytium.

The invasive syncytiotrophoblast is the only embryonic tissue in contact with maternal tissues at this stage. However, it has now been shown that the assumption is no longer true that only through the development of the extravillous trophoblast in the 5th week of pregnancy a further cell population comes into contact with maternal tissues. It is now becoming clear that first individual extravillous trophoblast cells come into direct contact with maternal tissues very early – at the time of the prelacunar phase (Moser and Huppertz 2017). However, further studies are necessary to clarify this issue.

The mononucleated trophoblast cells remaining behind the syncytiotrophoblast are now called cytotrophoblasts to distin-

guish them from the multinucleated syncytiotrophoblast. In the area of the invasion front, the invasive syncytiotrophoblast does not show a smooth surface, but is rather characterized by finger-shaped outgrowths that reach into the maternal tissues.

The syncytiotrophoblast no longer has the ability to divide and relies on fusion with the underlying cytotrophoblasts. The latter proliferate continuously, while the differentiating daughter cells eventually fuse with the syncytiotrophoblast, thus enabling its growth. Hence, the syncytiotrophoblast is a true syncytium and not a cell, it has no internal cell boundaries, and does not consist of individual syncytial units. Terms such as syncytiotrophoblasts (as plural) in a placenta or syncytial cells are therefore obsolete and should be strongly avoided.

1.2.2 Lacunar Phase (Days 8 to 13 after fertilization)

During this time, the syncytiotrophoblast grows around the entire embryo so that the placenta takes on a spherical shape at the end of this phase. At day 12 p. c. the embryo is completely grown into the endometrium of the uterus and surrounded by the placenta. The uterine epithelium at the implantation site is closed again, and a thin layer of endometrial connective tissue is found between the placenta and the uterine epithelium.

Around day 8 p. c., the first vacuoles appear in the syncytiotrophoblast, which merge into larger lacunae during the following days. These lacunae are separated from each other by larger structures of the syncytiotrophoblast, so-called trabeculae, which run from the embryonic side to the maternal side of the developing placenta. The fluid-filled lacunae gradually flow together to form a large space, which is subsequently called the intervillous space, the space between the villi. Maternal blood will later flow into this space to ensure the supply of the placenta and fetus with nutrients and oxygen.

Compartmentalization of the Placenta

The development of lacunae leads to compartmentalization of the placenta:

- The embryonic side of the placenta develops into the chorionic plate
- The trabeculae develop into the villous trunks, which on the one hand connect the chorionic plate with the decidual side of the placenta and on the other hand serve as a basis for villous development
- The developing lateral branches of the trabeculae develop into the free-floating villi
- The lacunae develop into the intervillous space
- The maternal side develops into the basal plate with parts of the uterine tissues.

1.2.3 Early Villous Phase (Days 13 to 28 p. c.)

Due to the proliferation of the cytotrophoblasts, a complete layer of these cells has formed under the syncytiotrophoblast. At about day 14 p. c. mesenchymal cells grow out of the embryoblast and form another layer underneath the cytotrophoblasts.

At the same time, the proliferation pressure of the cytotrophoblasts pushes the cytotrophoblasts to migrate into the syncytial trabeculae until they finally reach the maternal side of the placenta where they begin to invade the uterine tissues of the placental bed as extravillous trophoblasts (trophoblasts outside the villous structures).

Due to the migration of the cytotrophoblasts, the trabeculae have now developed into structures that are surrounded by the multinuclear syncytiotrophoblast and filled

with mononuclear cytotrophoblasts. These structures are called primary villi and are also found as branch-like structures extending from the trabeculae. The appearance of these primary villi marks the beginning of the villous phase of placentation.

Shortly thereafter, the mesenchymal cells of the extraembryonic mesoderm follow the cytotrophoblasts and also invade the trabeculae and primary villi, displacing the cytotrophoblasts from the core of the trabeculae and primary villi (Fig. 1.1). These cells penetrate almost to the maternal end of the trabeculae but do not come into contact with maternal tissues. A thick cushion of cytotrophoblasts forms between the mesenchymal cells and the maternal cells, which later is referred to as a trophoblastic cell column. As the cytotrophoblasts are displaced from the core areas of the trabeculae and primary villi by mesenchymal cells, secondary villi develop. Such villi are surrounded by two layers of trophoblast (syncytiotrophoblast and cytotrophoblast) and filled with a core of mesenchymal cells.

On day 18–20 p. c. vasculogenesis starts in the mesenchyme of the secondary villi, which thereby develop into tertiary villi (Fig. 1.1) (Demir et al. 2004). Hemangioblastic stem cells within the placental mesenchyme develop into endothelial as well as blood cells. Even at this early stage, placental macrophages are found to have developed within and from the placental mesenchyme. This early development of ves-

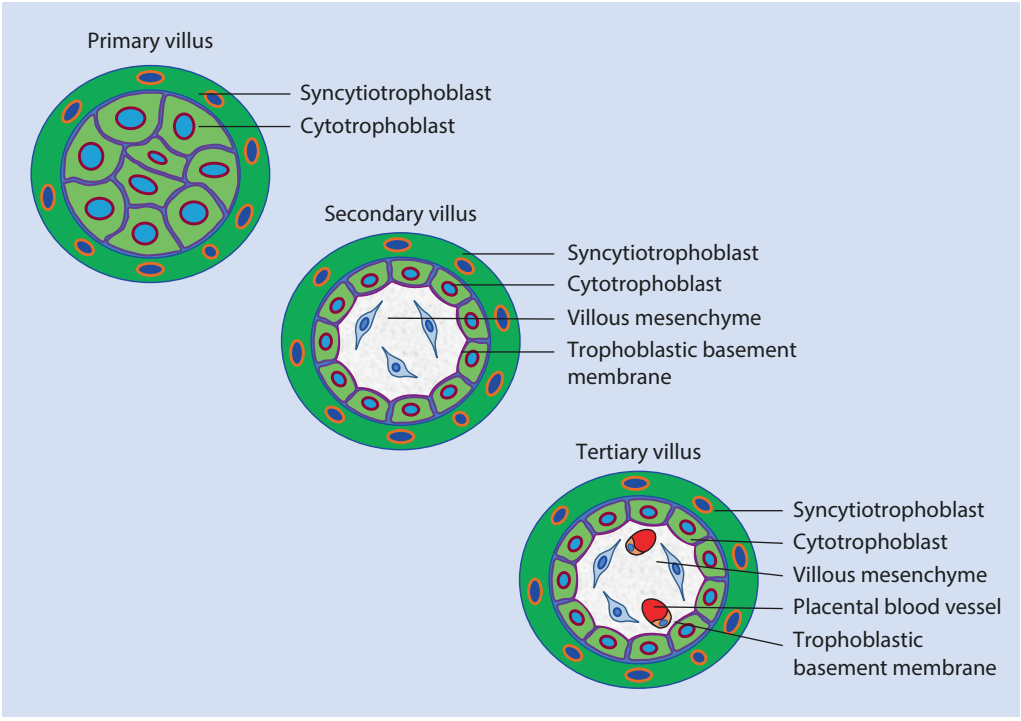

Fig. 1.1 Schematic representation of early villous development. The cytotrophoblast pushes into the syncytial sprout of a villus by increased proliferation in this area and gives rise to a purely trophoblastic primary villus. This is followed by the connective tissue stroma, which is separated from the trophoblastic covering by a basement membrane. Thus, a secondary villus is formed. Tertiary villi then develop with the development or sprouting of blood vessels into these villi. These tertiary villi are the villi that can then be further subdivided morphologically (Fig. 1.5)

sels and blood cells within the placenta proceeds independently of the development of vessels and blood cells in the embryo.

Hematopoiesis within the placenta is found during the first trimester, then the fetal liver takes over this function. The connection of the two vascular systems of placenta and embryo via the umbilical cord does not occur until around day 35 p. c., i.e. in the 7th week of pregnancy (Benirschke et al. 2006).

Due to a pronounced branching angiogenesis, almost all villi are vascularized tertiary villi by the end of the first trimester. One of the reasons for this massive angiogenesis is the intraplacental low oxygen concentration during the first trimester. The reasons for this are described and discussed in ▶ Sect. 1.5.6.

1.2.4 Villous Phase (Day 28 p. c. to end of pregnancy)

The growth of the villous tree follows a uniform principle from the first villus until the end of pregnancy:

On the surface of larger villi, the syncytiotrophoblast bulges outward and forms so-called syncytial sprouts. Below these syncytial sprouts, an increased proliferation of cytotrophoblasts begins, which results in filling of syncytial sprouts with cytotrophoblasts on the inside and thus becoming villous sprouts (= primary villi). Now the mesenchyme follows and grows into the developing villi (= secondary villi). Subsequently, angiogenesis within the villous stroma leads to the ingrowth of blood vessels into these regions (= tertiary villi). Due to a corresponding growth in length combined with a permanent sprouting of new villi, the placental villous tree grows continuously until the end of pregnancy (Benirschke et al. 2006).

Stratification of the Placental Barrier

This type of villous development results in the following stratification (from maternal to fetal) of the placental barrier, the barrier between maternal and fetal circulation (Fig. 1.2):

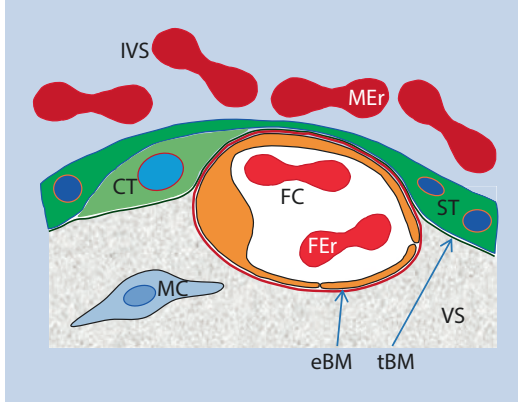

Fig. 1.2 Schematic representation of the placental barrier. The placental barrier is the barrier for the exchange of nutrients and gases between maternal and fetal blood. In this process, nutrients and gases need to transit through the syncytiotrophoblast as well as the endothelium of the fetal capillaries. Abbreviations: *CT* Cytotrophoblast, *eBM* Endothelial basement membrane; *FC* Fetal capillary, *FEn* Fetal endothelium, *FEr* Fetal erythrocytes, *IVS* Intervillous space, *MEr* Maternal erythrocytes, *MC* Mesenchymal cell, *ST* Syncytiotrophoblast, *tBM* Trophoblastic basement membrane, *VS* Villous stroma

- Syncytiotrophoblast: This is clearly visible in the first trimester and is characterized by an almost continuous row of nuclei. In the third trimester it thins out significantly to reduce the diffusion distance between maternal and fetal blood. At the same time, the syncytial nuclei are arranged in groups and relocated to specific areas to optimize diffusion at the sites of placental vessels.
- Cytotrophoblast: In the first trimester there is a complete layer of cells which lie directly under the syncytiotrophoblast and are separated from the villous stroma by a basement membrane. Due to the faster growth of the mesenchymal core of the villi compared to the proliferation rate of the villous cytotrophoblast, there is a separation of the latter cells during the further course of pregnancy. In particular, villous cytotrophoblasts are rarely found at the sites where placental vessels lie closely beneath the villous trophoblast. Thus, the diffusion distance is further reduced.
- Basement membrane of the villous trophoblast.
- Placental mesenchyme/connective tissue of the villi: Again, in the third trimester, there is a reduction of connective tissue at the sites where vessels directly approach the trophoblastic basement membrane. This is important to reduce the diffusion distance.
- Basement membrane of the placental endothelium: In the first trimester the placental blood vessels are not yet surrounded by a basement membrane. This develops only in the course of pregnancy.
- Endothelial cells: The final barrier of the placental barrier is found to be the endothelial cells of the placental vessels. The endothelium of the placental capillaries acts as a passive filter and limits the intercellular transfer of molecules (Eaton et al. 1993).

1.3 Placental Villi

1.3.1 General Histological Structure

The placental villi of the human placenta all share the same basic structure, although—as will be described in ▶ Sect. 1.4—different peculiarities of this structure are found during pregnancy. At the same time, the placental villi are not only the site of maternofetal and fetomaternal exchange, but also the site of most of the metabolic and endocrine activities of the placenta.

Histological Structure of the Placental Villi

The basic structure of the placental villi follows the following scheme (from outside to inside) (◘ Fig. 1.3):

- Villous syncytiotrophoblast
- Villous cytotrophoblast (also termed Langhans cells)
- Trophoblastic basement membrane
- Mesenchymal stroma/connective tissue, with
  - Local connective tissue cells such as mesenchymal cells, fibroblasts, myofibroblasts and smooth muscle cells
  - Migration-active cells such as macrophages (also termed Hofbauer cells)
  - Placental blood vessels (arteries, veins, capillaries, sinusoids)

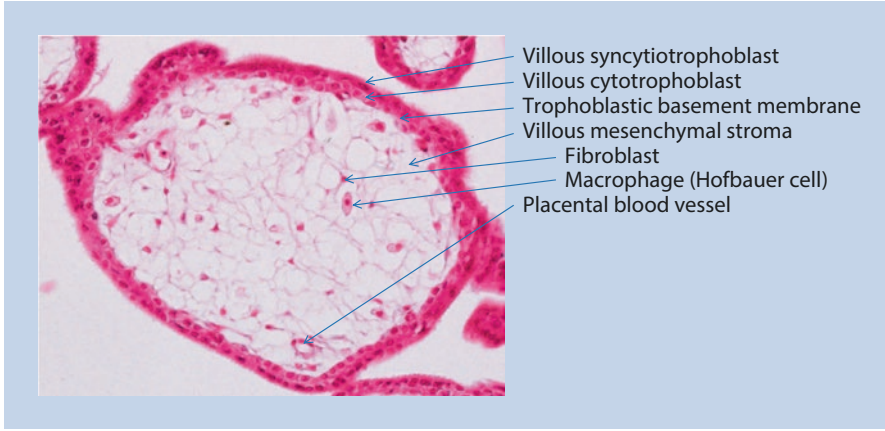

Fig. 1.3 Representation of the histological structures in a placental villus. Using the example of an immature intermediate villus from the first trimester, the most important histological structures of a villus are shown. At this stage, there is (1) a still double-layered trophoblastic coating of syncytiotrophoblast and cytotrophoblast with underlying basement membrane and (2) the villous stroma in the villous core with mesenchymal cells, fibroblasts and macrophages as well as placental blood vessels

1.3.2 Syncytiotrophoblast

The villous trophoblast, as an epithelial-like layer enclosing the villi, consists of two layers, the inner layer of the mononuclear cytotrophoblasts and the outer layer of the multinuclear syncytiotrophoblast. This syncytiotrophoblast was formed by fusion of the early trophoblast cells of the trophectoderm of the blastocyst. After implantation and throughout gestation, this syncytium is maintained by permanent fusion of the underlying cytotrophoblasts with the syncytiotrophoblast, thus introducing new cellular material into this syncytium.

In Vitro Culture and Syncytiotrophoblast

Here it must be made clear that there is no fusion of villous cytotrophoblasts with each other during pregnancy, otherwise there would be multiple layers of syncytia, which have not yet been described in humans. This is different to the situation in mouse and rat, where two layers of syncytia with one layer of cytotrophoblasts have been detected. In humans, however, only fusion events between single cells and the syncytium are found after implantation and during pregnancy, but not between two single villous cytotrophoblasts.

This must be taken into account when performing cell cultures with isolated cells. When villous cytotrophoblasts are isolated from a placenta and put into culture, fusion of these cells is often described as a criterion of good isolation and culture. However, if this does not occur in vivo, how can it occur in vitro? There are various explanations for this phenomenon; however, all have not yet been proven:

- During isolation, mononuclear fragments of the syncytiotrophoblast are also isolated and taken into culture. These and other, smaller portions of the syncytiotrophoblast may serve as an initial nuclei for fusion. Once a cytotrophoblast has fused with a fragment of the syncytiotrophoblast, it may be recognized as a "syncytium" by other cytotrophoblasts. This would then result in subsequent fusion between single cells and a 'syncytium'—similar to the in vivo situation.
- Due to the isolation, the villous cytotrophoblasts lose contact with the underlying basement membrane of the placental

villi. In vivo, this leads to a change in the phenotype of these cells from villous to extravillous trophoblasts. It has been shown many times that multinucleated extravillous trophoblast cells occur in the placental bed. Hence, it could be speculated that the villous cytotrophoblasts change their phenotype after isolation, become extravillous trophoblasts and subsequently fuse—similar to the in vivo situation.

Even though both approaches explain the behavior of villous cytotrophoblasts in culture, there is an urgent need for clarification of this behavior, since both approaches assume fundamentally different cell populations. This significantly affects the interpretation of the results of these cell cultures.

Structure and Function of the Syncytiotrophoblast

The syncytiotrophoblast is a true syncytium with an apical and a basal plasma membrane. There are no lateral cell borders in this system and thus there is only one syncytiotrophoblast in a placenta. Here it must be pointed out once again that the use of the plural for the syncytiotrophoblast of a placenta or the use of the term "syncytial cells" should be urgently avoided, as this indicates above all a lack of understanding of placental structures.

As the site of highest metabolic and endocrine activity in the placenta, the main role of the syncytium is maternofetal (and fetomaternal) transfer and its control. This includes but is not limited to (Benirschke et al. 2006):

- the active transport of amino acids and electrolytes such as sodium and calcium,
- the facilitated transport of glucose,
- the diffusion of water and gases, but also
- the synthesis of many hormones such as β-hCG (beta subunit of human chorionic gonadotropin) and
- the catabolism and resynthesis of proteins and lipids.

Histologically, the syncytiotrophoblast is recognized as the surface boundary of each villus. Its apical surface shows a light cover with microvilli to increase the surface area for exchange. Often, the nuclei are irregularly distributed and may occur in a row or very sporadically.

Functionally, the permanent fusion of the cytotrophoblasts with the overlying syncytiotrophoblast results in a constant transfer of fresh organelles, new enzyme systems and mRNA transcripts as well as whole nuclei into the syncytiotrophoblast. This helps to maintain the high metabolic activity of the syncytium, which is necessary for transfer as well as for secretory and endocrine functions.

The permanent influx of new nuclei into the syncytiotrophoblast leads to the fact that the nuclei in this system are of different ages and thus show different morphologies and chromatin contents. It has been shown repeatedly that the syncytial nuclei no longer exhibit proliferative activity. The presence of rare nuclei that may still be positive for certain proliferation markers can be explained by rapid differentiation and fusion after the last cell division of a villous cytotrophoblast. The different degrees of aging of syncytial nuclei are accompanied by different degrees of transcriptional activity of these nuclei. The freshly introduced nuclei still show a certain basic activity with regard to transcription, while this activity comes to an end in the course of the retention of the nuclei in the syncytiotrophoblast (about 3–4 weeks retention of a single nucleus within the syncytiotrophoblast).

Protrusions of the Syncytiotrophoblast

Histologically (and also pathologically), protrusions of different types and sizes are noticeable on the outside of the syncytiotrophoblast. The following structures can be distinguished here (◘ Fig. 1.4) (Kaufmann and Huppertz 2007):

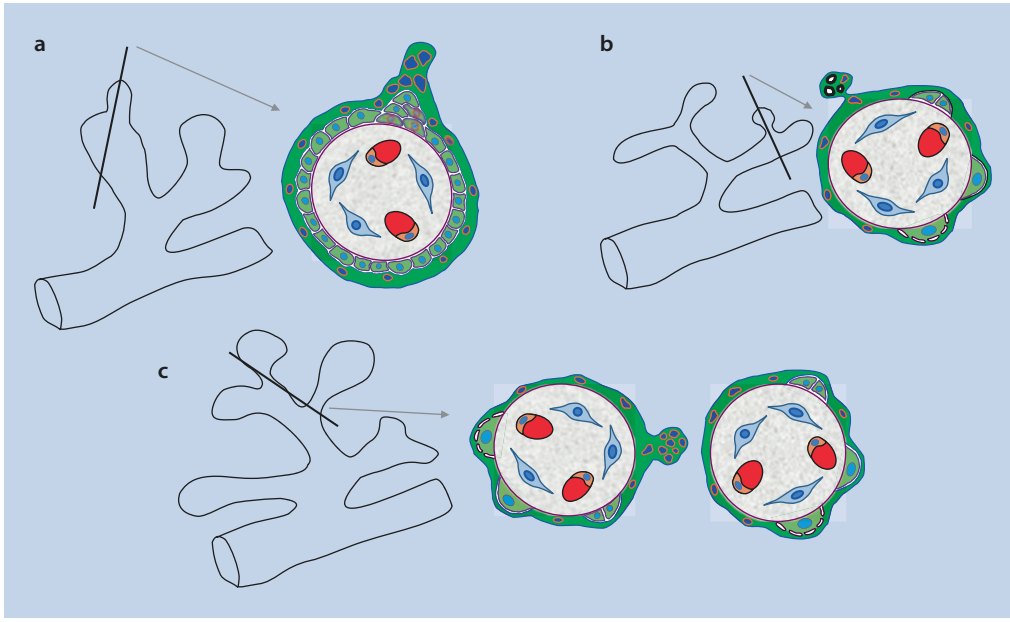

Fig. 1.4 Schematic representation of the protrusions of the syncytiotrophoblast. The "protrusions" of the syncytiotrophoblast visible in the histological sectional image can have different causes. **a** The development of additional villi proceeds via sprouting of primary villi from existing villi. These syncytial sprouts are found mainly in the first half of pregnancy and are true sprouts of the syncytiotrophoblast. **b** In the second half of pregnancy, syncytial knots are more commonly found. These structures are also outgrowths of the syncytiotrophoblast, but this time without involvement of the underlying cytotrophoblasts. Here, apoptotic material is packed into protrusions of the apical membrane of the syncytiotrophoblast and released into the maternal circulation as the final process of apoptosis in the villous trophoblast. **c** The Tenney-Parker changes are misinterpretations of flat sections through the syncytiotrophoblast. Thus, these are not protrusions, but artificial formations that arise from looking onto twodimensional sectional images of three-dimensional structures

- Syncytial/villous sprouts: These structures—as described in ▶ Sect. 1.2.4—serve the permanent growth of the villous tree of a placenta. Later, connective tissue parts of the villous stroma also push into these structures and thus lead to further branching of the villous tree. The syncytial sprouts are characterized by loosely grouped, large oval nuclei with little heterochromatin. The morphology of these syncytial nuclei suggests that the sprouts are growing structures.
- Syncytial knots: These structures serve to dispose of aged material and thus maintain homeostasis of the syncytiotrophoblast. The nuclei are densely packed and often show an irregular shape. They contain large amounts of heterochromatin up to the annular chromatin of late apoptotic nuclei. Occasionally, these nuclei can also be stained with the TUNEL assay, which shows single-strand breaks in DNA as a typical feature of late apoptosis.
- Syncytial knots are pinched off from the syncytiotrophoblast and thus released into the maternal blood flowing through the intervillous space. From there they are flushed into the maternal circulation via the uterine veins and thus enter the first capillary bed behind the placenta: the lungs. Here, these oligonuclear structures are degraded by pulmonary macro-

phages, leading to the fact that they are almost undetectable in peripheral blood of the mother.

- Flat sections through the syncytiotrophoblast/Tenney-Parker changes: The structure of the placenta was, and still is, worked out mainly by histological observation of two-dimensional sections, which is why the sections of branching villi and of transitions from one villus to another are often mistakenly regarded as syncytial sprouts or knots. These are flat sections through portions of the syncytiotrophoblast that look like protrusions of the syncytium in the sectional view. There is no uniform morphology of syncytial nuclei here, as the flat sections can involve any area of the syncytiotrophoblast.

Because some pathologies change the branching pattern of placental villi and thus the frequency of flat sections, these structures have often been described and named after the names of the two first describers: Tenney-Parker changes (Tenney and Parker 1940). Although not true protrusions of the syncytiotrophoblast, these structures can still be used as indicators of changes in villous structure. Quantification to classify and differentiate between pathological cases and healthy placentas is therefore quite useful. However, differentiation between Tenney-Parker changes and syncytial knots in sections of a delivered placenta is extremely difficult. Often both together are wrongly described as syncytial knots.

1.3.3 Villous Cytotrophoblast (Langhans Cell)

The epithelial layer of the villous trophoblast not only consists of the multinuclear syncytiotrophoblast, but includes a layer of mononuclear cytotrophoblasts as a second layer under the syncytium. These mononuclear villous trophoblast cells have positioned themselves under the syncytiotrophoblast early in placental development and remain there until the end of pregnancy.

They mainly serve the continuous supply of fresh material for the overlying syncytiotrophoblast. Thus, many proliferating cells are found in this cell layer, and mitotic figures can be observed there as well. The postproliferative daughter cells within this layer start their differentiation, thereby significantly increasing their transcription and translation rates and thus store large amounts of mRNA and proteins, which are then transferred into the syncytiotrophoblast during the subsequent syncytial fusion event.

Morphologically, villous cytotrophoblasts are characterized by being roundish in section, sometimes polygonal. Their nuclei are large and weakly stained, with little heterochromatin.

The importance of maintaining the syncytiotrophoblast for the function of the villous cytotrophoblasts becomes apparent when the syncytium is damaged. Breaks in the syncytium are resealed by fibrin deposits initiated by maternal blood (fibrin-type fibrinoid, perivillous fibrin deposition). If such an area is so large that underlying villous cytotrophoblasts lose contact with the syncytiotrophoblast, these single cells start their differentiation towards the extravillous trophoblast phenotype. Thus, they are no longer available as proliferating progenitor cells for the syncytiotrophoblast.

While in the first trimester there is still an almost complete layer of villous cytotrophoblasts under the syncytiotrophoblast, this layer thins out in the course of pregnancy, so that in the third trimester only individual cells are found under the syncytium. This is mainly due to an enormous expansion of the volume of the villous stroma, rather than a decrease in the proliferation activity of the cytotrophoblast. This can be seen from the fact that the ratio of nuclei in cytotrophoblasts to nuclei in the

syncytiotrophoblast is about 1:9, regardless of whether this value is determined in the period 13–16 weeks of gestation or 37–41 weeks of gestation (Mayhew et al. 1999).

1.3.4 Villous Stroma

The connective tissue stroma of the placental villi develops from the extraembryonic mesoderm of the early villous phase (▶ Sect. 1.2.3). The initially predominant fixed connective tissue cells, the undifferentiated mesenchymal cells, differentiate into fibroblasts, which are the most widespread cells in the stroma by the end of pregnancy. At the end of pregnancy, the mesenchymal cells appear only in small areas, especially directly under the basement membrane of the villous trophoblast in stem villi. Around larger vessels, the fibroblasts further differentiate into myofibroblasts, which become smooth muscle cells in the media of the vessels.

The fixed connective tissue cells of the placental stroma produce many connective tissue fibers, including type I and III collagens and fibronectin. These fibers serve to increase the mechanical stability of the villi. This is particularly necessary against the background of the permanent movement through the two blood flows of mother and child in the placenta.

Macrophages, also called Hofbauer cells in the placenta, make up the majority of free connective tissue cells. These macrophages either originated directly from mesenchymal progenitor cells in the placenta or were recruited from the fetal circulation. To date, no marker is known that can distinguish these two subtypes of Hofbauer cells. Hofbauer cells secrete a large number of growth factors and are also involved in the differentiation of the villous trophoblast and in vasculo- and angiogenesis in the placenta (Demir et al. 2004).

In addition to Hofbauer cells, a few plasma and mast cells are also found in the villous stroma.

1.3.5 Placental Blood Vessels

Embedded in the villous stroma are the placental vessels. Starting from the two umbilical arteries, the fetal blood flows through the chorionic plate arteries into the arteries of the villi. There the arterial vessels branch further until, starting from small arterioles, the blood flows through the capillary bed of the placenta. This capillary bed with a surface of more than 10 m^2 serves the exchange between mother and child. At the time of delivery, the capillary bed is mainly found in specific types of villi (Terminal villi; ▶ Sect. 1.4.5). In such villi, the blood vessels are mostly found in direct vicinity of the villous trophoblast. The return flow to the fetus then takes place via the venous leg, which runs via the villous veins into the chorionic plate veins and from there into the one umbilical vein.

The contractile cells around the large placental vessels are clinically important. Reduced fetoplacental perfusion, which can be visualized by Doppler ultrasound of the umbilical arteries, is closely associated with reduced fetal growth (FGR, “fetal growth restriction”, or IUGR, “intrauterine growth restriction”), which can lead to intrauterine fetal death (Krebs et al. 1996).

Because placental vessels have no autonomic innervation (as the entire placenta has no innervation), regulation of placental blood flow occurs via local and systemic factors that control blood flow along with the anatomical arrangement and fetal cardiac output.

1.4 Architecture of the Villous Tree

Even if the presentation of the architecture of the placental villous tree seems like an academic exercise, it has been shown that this architecture has clinical relevance. The significant changes in villous structure and types in conjunction with changes in the pla-

cental vasculature are clearly seen in FGR and are associated with placental insufficiency.

During pregnancy, a total of five types of villi have been defined based on circumference/diameter, stromal characteristics, and vascular structures (Benirschke et al. 2006; Baergen 2011) (Fig. 1.5).

Over the course of pregnancy, villous types of different appearance and functions develop. Thus, each stage of pregnancy can be assigned to a specific set of villous types and functions (Fig. 1.6).

1.4.1 Mesenchymal Villi

Mesenchymal villi (Fig. 1.5a) are the first villi to develop as tertiary villi and thus have all three major components of a villus: villous trophoblast, villous stroma, and placental blood vessels. This occurs at about day 20 p. c. Thus, mesenchymal villi are the precursors of all other types of villi. In the first and second trimesters, mesenchymal villi differentiate into immature intermediate villi (▶ Sect. 1.4.2), whereas in the third trimester they develop predominantly into

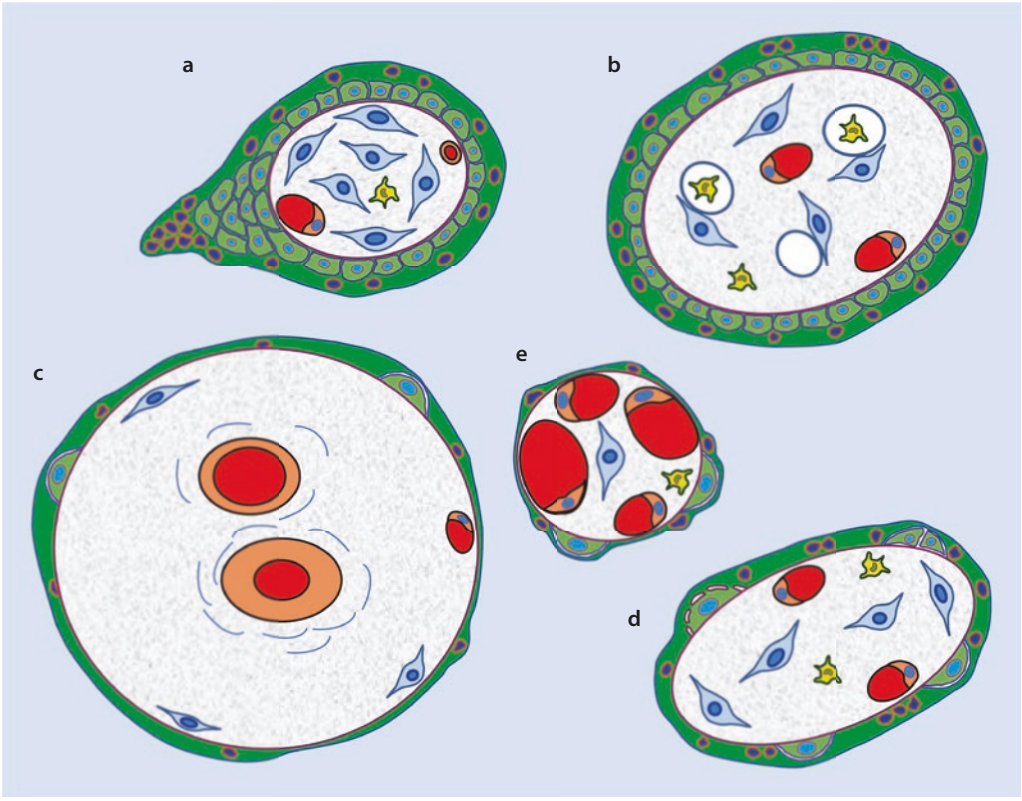

Fig. 1.5 Schematic representation of the different types of villi. The five different types of villi of the human placenta can be distinguished on the basis of morphological criteria. **a** Mesenchymal villus with many mesenchymal cells, small vessels and a bilayered trophoblast. **b** Immature intermediate villus with a loose stroma, small vessels and stromal channels. Again, the villous trophoblast is bilayered. **c** Large stem villus with two centrally located vessels (artery and vein), dense fibrous stroma, and only few sporadic cytotrophoblasts. **d** Mature intermediate villus with loose connective tissue, small vessels and few cytotrophoblasts. **e** Terminal villus with sinusoids and capillaries lying closely under a thin syncytiotrophoblast

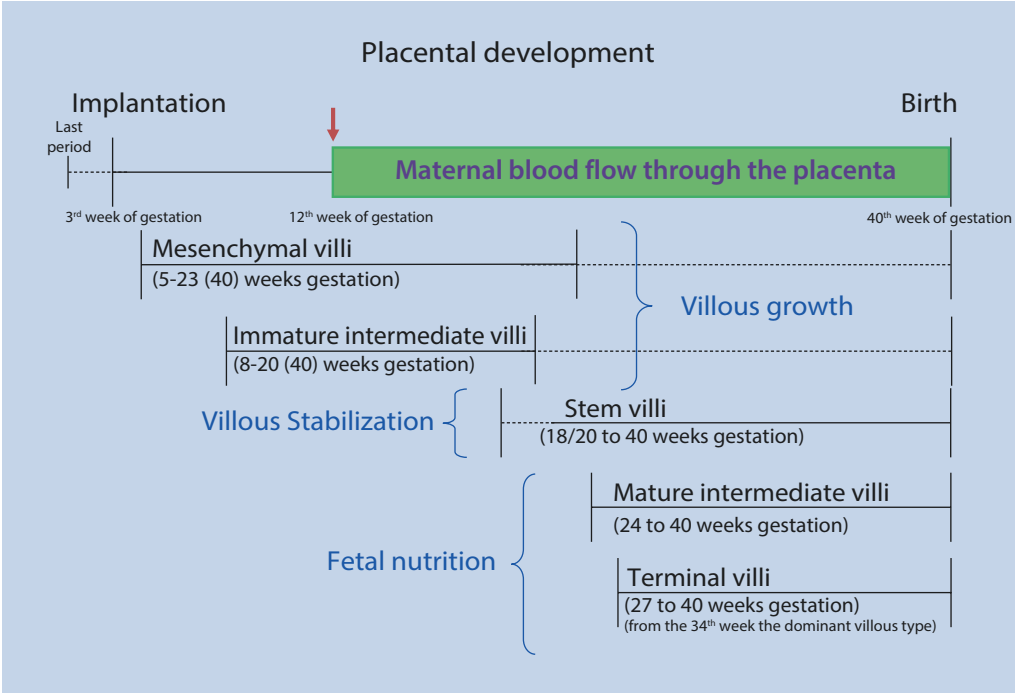

Fig. 1.6 Representation of the development of villous types during pregnancy. Over the course of pregnancy, the change in villous types becomes obvious. At the beginning, mesenchymal villi and immature intermediate villi are necessary for the growth of the villous trees, but around mid-gestation, the villous structures are increasingly stabilized by the developing stem villi. With the second half of pregnancy, mature intermediate villi and finally terminal villi develop for the adequate nutrition of the fetus until birth

mature intermediate villi (▶ Sect. 1.4.4). While they are the predominant type of villi at the beginning of pregnancy, mesenchymal villi in a term placenta are only found in very small areas in the centers of villous trees and account for less than 1% of the volume of all villi.

Mesenchymal villi are about 40–80 μm in diameter, have a distinct bilayered villous trophoblast, and have a stromal core with few extracellular fibers such as collagen type I, few fibroblasts and Hofbauer cells, and first small blood vessels. However, there are many mesenchymal cells in the stroma. Due to the high proliferation rate of mesenchymal cells and cytotrophoblasts in this type of villi, mesenchymal villi are the villi with the highest proliferation rate.

1.4.2 Immature Intermediate Villi

Immature intermediate villi (Fig. 1.5b) are the first type of villi differentiating from mesenchymal villi, starting around the 8th week of gestation. Through massive growth and further differentiation, this type of villus becomes the predominant type of villus between weeks 14 and 20 of gestation. In a term placenta, however, this type of villus, similar to the mesenchymal villi, almost completely disappears and only accounts for about 5% of the volume of all villi.

Like mesenchymal villi, immature intermediate villi have a distinct bilayered villous trophoblast. However, they are significantly larger with a diameter between 100 and 300 μm. Their stromal core shows loose reticular

connective tissue with few matrix components, small blood vessels and a large number of Hofbauer cells. These cells stand out in immature intermediate villi because these villi have a distinctive feature not found in any other type of villus: stromal channels. These are fluid-filled cavities that run along the longitudinal axis of the villi. They are surrounded by fibroblasts but have no connection to the vasculature and also show no demarcation by endothelial cells. Hofbauer cells are often found in these channels, hence easily recognizable in this type of villus, although macrophages are present in all types of villi.

The immature intermediate villi are a type of villus that is only found temporarily, as they develop from mesenchymal villi and subsequently differentiate into stem villi (▶ Sect. 1.4.3). This explains the marked decrease in the number of this type of villus at the beginning of the second half of pregnancy. Immature intermediate villi are often mistakenly called edematous villi because the presence of stromal channels is misinterpreted. The retention of this type of villus in a mature placenta is not due to edematous changes in the placenta, rather it is a clear indication of a lack of villous maturation and is associated with fetal growth restriction.

1.4.3 Stem Villi

Stem villi (◘ Fig. 1.5c) are formed by further differentiation of the immature intermediate villi and appear from the 18th/20th week of pregnancy. They are the largest villi in a placenta and range in diameter from 100 µm to several millimeters and can be seen with the naked eye. Due to their size, they account for about 20-25% of the volume of all villi in a term placenta. They extend as large trunks from the chorionic plate into the intervillous space. Many of the first branches of these large trunks are again stem villi (second generation). Also the anchoring villi that connect the placenta to the basal plate are stem villi.

Even if the caliber of this type of villus shows clear differences, the morphological characteristics are relatively clear. The villous trophoblast is no longer very pronounced in stem villi and is often replaced by fibrin deposits. The stroma is filled with many fibers of the extracellular matrix to account for the mechanical stability of these villi. The number of fibroblasts and Hofbauer cells is relatively low, and a few mast cells are also found. On the other hand, the very large villi are characterized by the presence of two central vessels (one artery, one vein). Around the media of these vessels contractile cells (myofibroblasts) are found, which represent a placenta-specific perivascular system for the local regulation of the vessel width and hence the blood flow through the villous trees. In addition, there are small vessels at the periphery of stem villi.

The function of the stem villi is the mechanical stabilization of the villous trees of a placenta, and here they function similarly to the trunk of a tree. Their function is not the exchange of nutrients and gases but rather to transport nutrient and oxygen-rich blood from the villous trees to the fetus (large vein) and to transport nutrient and oxygen-depleted blood from the fetus to the villous trees (large artery) to the exchange areas of the terminal villi (▶ Sect. 1.4.5).

1.4.4 Mature Intermediate Villi

At the beginning of the third trimester (approximately from the 24th week of gestation), the mesenchymal villi no longer differentiate into immature intermediate villi, but into a new type of villus, mature intermediate villi (◘ Fig. 1.5d) with a diameter of 80–120 µm. While the differentiation into immature intermediate villi was crucial

for the general growth of the villous trees, the differentiation into mature intermediate villi is mandatory for the increased development of the exchange surface of the placenta. The latter is required to meet the significantly increased needs of the fetus in terms of nutrient and oxygen uptake. The mature intermediate villi make up about 25% of the volume of all villi in a term placenta.

Mature intermediate villi are long and narrow, possessing a loose connective tissue stroma and many, often marginal, small vessels, predominantly capillaries, but also small terminal arterioles and venules.

1.4.5 Terminal Villi

Terminal villi (Fig. 1.5e) are the last type of villi to differentiate during pregnancy. They arise from increased growth in length of the capillaries in mature intermediate villi, which bulge outwards as a result of this growth, thus generating new villi. Terminal villi are therefore not formed by the differentiation of precursor villi like intermediate and stem villi, but from the 27th week of pregnancy by protrusions starting from mature intermediate villi. The function of terminal villi is to facilitate exchange between mother and fetus across the placental barrier. Therefore, this type of villi accounts for about 40% of the villous volume in a term placenta. Since they are terminal protrusions, their length can be given as up to 200 µm, with a width of 50–100 µm.

The trophoblastic coating of terminal villi varies in thickness. Where capillaries lie directly beneath the villous trophoblast, the thickness of the syncytiotrophoblast is reduced to a few micrometers and nuclei are absent, while cytotrophoblasts are very rarely found in these areas. Here the basement membrane of the capillaries comes into direct contact with the trophoblast basement membrane. Thus, at these sites the placental barrier has its least thickness with (1) the syncytiotrophoblast, which is minimally thick, (2) the fused basement membranes of trophoblast and capillary, and (3) the endothelium. This unit is also referred to as the "vasculosyncytial membrane" in the literature. Here the diffusion distance is reduced to 0.5–2 µm.

In areas without direct connection to underlying vessels, the syncytiotrophoblast is much thicker, nuclei often lie together in groups, and cytotrophoblasts are also found here. In terminal villi, the capillaries in the stroma dilate, becoming sinusoids to increase the exchange area and slow blood flow in these areas. Thus, the stroma of terminal villi is characterized by capillaries and sinusoids, which may account for more than 30% of the stromal sectional area of a terminal villus.

The number of terminal villi adapts to the microenvironment in the placenta. In mothers with chronic anemia or in mothers living at high altitude, less oxygen reaches the placenta. The placenta in the second and third trimester responds by increasing its exchange surface area. Increased proliferation of endothelial cells in the mature intermediate villi leads to increased formation of terminal villi. On the other hand, the opposite picture is seen in growth-restricted fetuses with insufficient circulation through the placenta (AEDF, "absent end-diastolic flow", or REDF, "reversed end-diastolic flow", in the umbilical arteries). In these cases, too little oxygen is transported from the placenta to the fetus. This results in an increase in oxygen concentration within the placental intervillous space compared to the normal weight fetus. Again, the placenta reacts, this time with a reduction in surface area and regression of terminal villi.

1.5 Extravillous Trophoblast

During the early villous phase (about day 15 p. c.), mononuclear trophoblast cells come into direct contact with maternal tissues for the first time (▶ Sect. 1.2.3). Because of

their location outside the villous structures, they are called "extravillous trophoblasts". All trophoblasts that do not function as epithelial-like cells of the villi are grouped under the term extravillous trophoblast. This includes trophoblasts in the chorionic plate, in the fetal membranes, in the basal plate and placental bed, and in villous areas where the trophoblastic covering is no longer present. All these extravillous trophoblasts have in common that they express histocompatibility antigen G (HLA-G) and can thus be distinguished from the villous trophoblast (McMaster et al. 1995). HLA-G is mainly used in (immuno)histological examinations as an unambiguous marker for the extravillous trophoblast.

After penetrating the syncytiotrophoblast in the early villous phase, the extravillous trophoblasts form multilayered columns of cells at these sites, the so-called trophoblast cell columns. Within these cell columns, there is a gradient starting from the villous trophoblast on the basement membrane of the anchoring villus to the distally located invasive extravillous trophoblasts. The cells adhering to the basement membrane show proliferative activity, which is lost in the next layers. The postproliferative daughter cells are pushed distally by proliferative pressure and change their phenotype.

The cells start to express HLA-G and also change their expression pattern of matrix proteins, matrix-degrading proteases and integrins to start their invasion into the maternal tissues of the uterus. The expression pattern and the invasive behavior of extravillous trophoblasts are very similar to those of invasive tumor cells. However, they differ from tumor cells in one crucial aspect: Extravillous trophoblasts that exhibit invasive behavior have lost their proliferative activity. Should they be flushed into the maternal bloodstream, metastasis cannot occur because these cells no longer exhibit the ability to divide. At the same time, this also determines the depth of invasion, since it depends directly on the lifespan of these cells and cannot be transferred by proliferation to daughter cells, which could then expand the depth of invasion.

Invasion of extravillous trophoblasts is not limited to early pregnancy but is a continuous process until the end of pregnancy. The area of the uterine wall that lies beneath the placenta and is invaded by extravillous trophoblasts is called the placental bed. The portion of the decidua that is invaded is named decidua basalis. The portion of the decidua basalis that is delivered with the placenta at birth is called the basal plate.

▪ Populations of the Extravillous Trophoblast

Trophoblast invasion serves multiple purposes and is implemented by distinct populations of extravillous trophoblasts (Fig. 1.7):

- Interstitial trophoblast: origin of all other populations of the extravillous trophoblast in the placental bed, starting from the trophoblast cell columns, this is the population found in the connective tissue of the decidua basalis and in the myometrium; function: adhesion of the placenta to the uterine wall.
- Endoglandular trophoblast: erosion and opening of the uterine glands to allow histiotrophic nutrition of the embryo during the first trimester.
- Endovascular trophoblast: So far, only the extravillous trophoblast invading the spiral arteries was called endovascular trophoblast. Today, it appears that also the uterine veins are invaded. Therefore, the nomenclature needs to be updated here.
  - Endoarterial trophoblast: erosion, opening and remodeling of the uterine spiral arteries to provide hemotrophic nutrition of the

1

fetus during the second and third trimesters.
- Endovenous trophoblast: erosion and opening of the uterine veins to ensure the return of maternal blood from the intervillous space of the placenta back into the maternal system.

- Endolymphatic trophoblast: Also the uterine lymphatic vessels are eroded by invading extravillous trophoblasts. The purpose of this erosion is not yet clear.

1.5.1 Interstitial Trophoblast

The cells that initially invade the uterine connective tissue are called interstitial extravillous trophoblasts and are thus precursors of all other types of extravillous trophoblast in the placental bed. These cells invade down into the inner third of the myometrium and thus do not reach the outer wall of the uterus in a normal pregnancy.

On their way through the uterine interstitium, the interstitial trophoblasts secrete extracellular matrix proteins, which in their entirety are named matrix-type fibrinoid

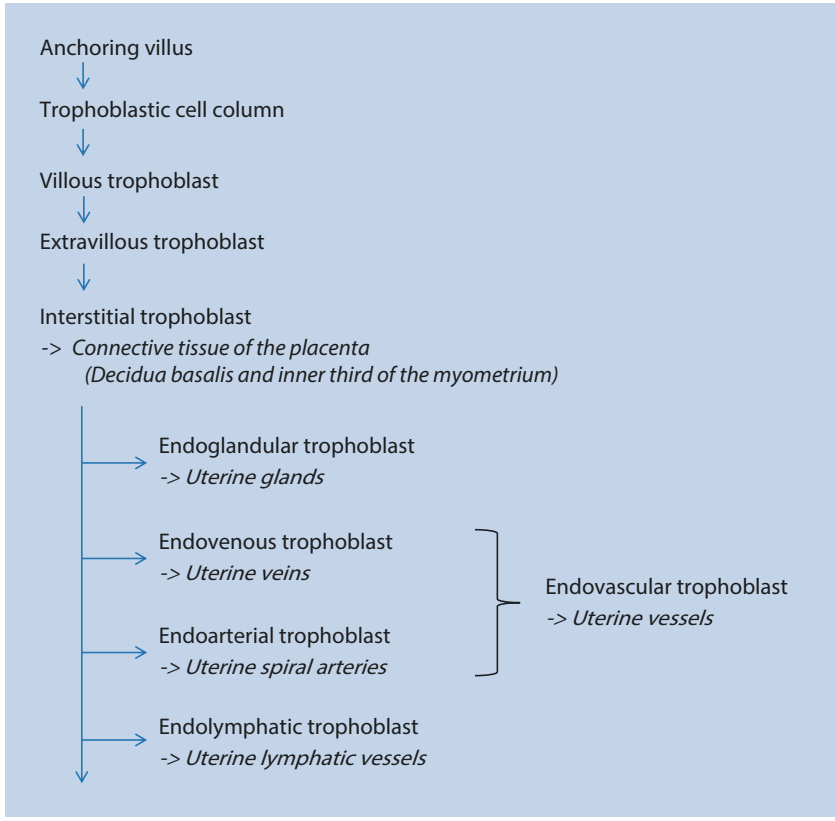

Fig. 1.7 Representation of the differentiation of the extravillous trophoblast. Starting from the cell columns of the anchoring villi, extravillous trophoblasts differentiate and migrate into maternal uterine tissues. During this process, they reach the luminal structures of the uterine wall. It is now apparent that extravillous trophoblasts invade all luminal structures in the uterus, although an explanation of why this occurs is still pending, at least in the case of lymphatic vessels. At the same time, the nomenclature had to be changed to represent the invasion of all luminal structures in a meaningful way

(Kaufmann et al. 1996) and serve as glue for anchoring the placenta to the uterine wall.

The population of the interstitial trophoblast is morphologically and functionally very heterogeneous. Different phenotypes can be distinguished with respect to invasion behavior, contact to maternal cells, and secretion of and attachment to extracellular matrix components. So far, the molecular basis for these different phenotypes is missing; however, there are corresponding explanations for the different trophoblast phenotypes in the mouse (Simmons et al. 2007). A better understanding of the molecular basis of trophoblast phenotypes in humans would help to understand the deviations from them in specific diseases during pregnancy. These include premature placental abruption, fetal growth restriction (FGR) and early preeclampsia.

Typically, three phenotypes can be distinguished morphologically and functionally in the population of interstitial trophoblasts:

1. Large polygonal cells

   Morphologically, these cells are large, polygonal cells with a single nucleus that is large, irregularly shaped, and clearly stainable. No other phenotype of all trophoblasts shows stronger immunohistochemical staining for cytokeratin 7. At the same time, these cells are always negative for proliferation markers such as Ki-67.

   This phenotype was previously described as X cells and is the cell type that predominantly secretes matrix-type fibrinoid (Kaufmann et al. 1996). The matrix contains components of basement membranes such as laminins and type IV collagen, but also fibronectin and vitronectin and an amorphous ground substance containing heparan sulfate (Huppertz et al. 1996). These large cells surround themselves with this matrix and anchor to this matrix with appropriate integrins (alpha5/beta1, alpha1/beta1 and alpha-v/beta3/5 integrins). Thus, they are rarely in direct contact with maternal cells and can be detected in the uterus even decades after the last pregnancy.

   The above characteristics of these cells do not indicate a strong invasiveness of these cells. Rather, this phenotype is thought to perform the task of attaching the placenta to the uterine wall. This also explains the strong expression and secretion of matrix-type fibrinoid by these cells.

   In early pregnancy (week 9–12), this phenotype accounts for about 45% of all interstitial trophoblasts. This proportion increases during pregnancy to 69% (week 16–24) and peaks at term at 89% (week 31–39) (Benirschke et al. 2006). These cells are relatively evenly distributed over the basal plate and the depth of invasion, reaching the inner third of the myometrium.

2. Small spindle-shaped cells

   There are only few descriptions of this phenotype so far. One of the reasons for this could be that these cells are often overlooked when looking at tissue sections due to their morphology, which is very small and spindle-shaped. This phenotype is only moderately immunoreactive for cytokeratin 7 and is—like all extravillous trophoblasts—negative for proliferation markers such as Ki-67. Structurally, this phenotype is characterized by small, oval nuclei and its elongated, almost filamentous cell body, which is oriented in the direction of invasion and surrounded by only little matrix.

   These small cells secrete little extracellular matrix, which consists mainly of cellular and oncofetal fibronectin. At the same time, these cells express only interstitial integrins (such as alpha5/beta1

and alpha-v integrins). This combination of and interaction between interstitial integrins and oncofetal fibronectins is an essential mechanism for trophoblast invasion (Huppertz et al. 1996). This phenotype is thought to be highly invasive.

Contrary to the large polygonal phenotype, the number of the small phenotype is reduced during pregnancy. While its proportion is about 55% during early pregnancy, it is only 31% at mid gestation and further reduced to 11% of the interstitial trophoblasts at term (Benirschke et al. 2006). This could be an indication of the decreasing invasive activity of the extravillous trophoblast and the decreasing proliferation activity of the trophoblast cell columns. These small cells can be visualized over the entire invasion area and thus show the same distribution pattern as the large polygonal cells.

3. Multinuclear giant cells

The difference to the other phenotypes of the interstitial trophoblast is that these cells have more than one and up to ten nuclei. These nuclei are irregularly shaped and have different sizes. Thus, these cells have a much larger volume than the other phenotypes, with cell diameters between 50 and 100 μm (Benirschke et al. 2006). The division-incompetent multinuclear giant cells are either mostly immunonegative for cytokeratin 7 or show only small areas of immunoreactivity. Thus, they can be easily overlooked in a superficial view of trophoblast invasion. Although syncytial fusion of extravillous trophoblasts has never been observed, it is generally accepted that these giant cells result from fusion of mononuclear single cells of the interstitial trophoblast. This phenotype is found predominantly in the depth of the placental bed, at the boundary between the decidua and the myometrium. Its share in the total number of interstitial extravillous trophoblasts is very small.

1.5.2 Endoglandular Trophoblast

In recent years, the picture of trophoblast invasion has changed significantly. So far, only two types of extravillous trophoblast have been described, the interstitial trophoblast and the trophoblast surrounding and invading spiral arteries. Today, the picture has been supplemented by several new populations of the extravillous trophoblast (Moser et al. 2010, 2015). These include the endoglandular trophoblast, which, differentiating from the interstitial trophoblast, reaches and erodes the uterine glands early in gestation.

Secretion products from the uterine glands in the intervillous space of the placenta in the first trimester of pregnancy were described earlier, but without knowing the mechanisms by which the secretion products entered this space (Burton et al. 2002, 2007). Only the demonstration that extravillous trophoblasts not only migrate past the uterine glands but specifically invade them, clarified how histiotrophic nutrition of the embryo can be achieved before the start of maternal blood flow into the placenta (Moser et al. 2010). Infiltration into the glands and replacement of the glandular epithelium results in opening of uterine glands by endoglandular trophoblasts, creating the pathway to connect these luminal structures of the uterus to the intervillous space of the placenta (Moser et al. 2010).

Recent studies expand this picture: close examination of archival material from the earliest placental stages shows that the endoglandular trophoblast is the first population of the extravillous trophoblast to erode uterine luminal structures during pregnancy (Moser and Huppertz 2017).

1.5.3 Endovascular Trophoblast

For decades it was assumed that only the spiral arteries were opened and remodeled by extravillous trophoblasts. Invasion into uterine veins was always excluded. This did not take into account that maternal blood flowing into the placenta via the opened spiral arteries needs to be transported back into the maternal bloodstream via maternal veins in the uterus. The discovery of trophoblast invasion into uterine veins was therefore not unexpected.

However, this fact also implies that the term "endovascular trophoblast" no longer refers only to cells that invade arteries (Kaufmann et al. 2003). Meanwhile, this term must be broadened to serve for all extravillous trophoblasts that invade vascular structures of the mother (i.e., arteries and veins). Thus, new terms need to define specifically invading trophoblast populations. All extravillous trophoblasts that invade vascular structures are now grouped together as endovascular trophoblasts. The cells that invade arteries are called endoarterial trophoblasts, while the cells that invade veins are named endovenous trophoblasts (Moser and Huppertz 2017).

Endoarterial Trophoblast

Starting from the interstitial trophoblast, a specific population of trophoblasts reaches the uterine spiral arteries, penetrates their media and also their endothelium and thus enters the lumen of these vessels. Contrary to some notions, this endoarterial trophoblast (formerly endovascular trophoblast) must penetrate the vessel wall from the outside to reach the interior of the vessels (intravasasion). The notion that these trophoblasts start to erode the walls of the vessels from within must be abandoned, since no cells, and thus no basis, exist for such extravasasion. It is quite conceivable that endoarterial cells, once invaded from the interstitium into the vessel, migrate along the luminal side of the endothelium of the vessel wall and subsequently re-invade the vessel wall (Kaufmann et al. 2003).

The main task of the endoarterial trophoblast is not only to open the spiral arteries towards the intervillous space of the placenta, but above all the conversion of the arteries into dilated tubes, which are no longer subject to maternal vascular control. This, however, refers only to the terminal parts of these vessels lying furthest towards the placenta in the region of the decidua. Thus, invasion into arteries is the only trophoblast invasion in which, in addition to the opening of a luminal structure, the wall of these structures is also remodeled. In the case of invasion into glands, veins and lymphatic vessels, these structures are only opened, but the walls are not further remodeled.

The transformation of the spiral arteries can be divided into three stages:

1. Maternal factors induce initial changes in the uterine spiral arteries very early in pregnancy, long before the first extravillous trophoblast has reached these vessels. The vessels begin to dilate due to decreasing vascular smooth muscle cell organization and altered endothelial cell morphology. It appears to be maternal uterine immune cells that are responsible for these changes. They accumulate in the decidua around these vessels and play an active role in the early stages of remodeling of these vessels. In this process, they secrete growth factors and matrix metalloproteinases that degrade the extracellular matrix of the vessel walls.
2. Once the vessel wall is disorganized and the vessel is slightly dilated, endoarterial trophoblasts from the connective tissue of the decidua invade and penetrate the vessel wall. The cells that have migrated into the vessel lumen form a plug that closes these vessels, preventing maternal blood from flowing into the placenta (Weiss et al. 2016).

3. Endoarterial trophoblasts subsequently also invade the vessel wall in the deeper areas of the vessels, and some of the cells that have already invaded will probably also migrate down the endothelium and may invade into the vessel wall again. Thus, the vessel walls are further invaded from the outside and inside and thus disorganized. This leads to further dilatation of the vessels, which in turn results in a vessel diameter many times larger than that of the original vessel. Reduced smooth muscle cell activity and loss of elastic fibers further contribute to the increase in vessel diameter.

Endovenous Trophoblast

Invasion of extravillous trophoblasts into uterine veins has only been described for the first time in 2017 (Moser et al. 2017). As outlined in ▶ Sect. 1.5.3, there must be a mechanism that also connects the uterine veins to the intervillous space to allow maternal blood flow from the placental intervillous space back into the mother's bloodstream. Thus, the new concept of venous invasion does not challenge the already existing concept of endoarterial (formerly endovascular) trophoblast invasion, but extends it in a useful way.

While invasion into uterine spiral arteries involves not only opening and connection to the intervillous space, but also remodeling of the vessel wall and plug formation in the lumen of these vessels, only opening and connection to the intervillous space seem to be crucial for invasion of the uterine veins—as is the case for the glands. Endovenous trophoblasts are found in the immediate vicinity of the veins, replacing the endothelial cells of the veins, and also in the lumen of these vessels. However, so far there is no evidence of remodeling of the vessel walls and no evidence of occlusion of the lumen. Neither would make sense functionally, since reflux into the maternal system needs to occur very early in pregnancy.

1.5.4 Endolymphatic Trophoblast

In the meantime, there are first indications that the uterine lymphatic vessels are also eroded. Two publications from 2017 show first data on this (He et al. 2017; Windsperger et al. 2017). Why invasion into these luminal structures of the uterus occurs is still unclear. It is possible that the extravillous trophoblast is more invasive than previously thought and invades all luminal structures that lie within its expansion range.

1.5.5 General Considerations on Trophoblast Invasion

The invasive potential of the extravillous trophoblast is optimally regulated. Thus, only few malignant transformations of the extravillous trophoblast have been described so far. Chorio carcinomas arise from villous but not from extravillous trophoblasts. For further information, see Sect. 3.2 "Histopathology of the placenta".

Excessive Invasion

Alterations on the maternal side, such as uterine scars, can lead to dramatic changes in invasion. In such cases, extravillous trophoblasts may invade deeper than usual (Jauniaux et al. 2016):

- Placenta accreta: Here the decidua is partially or completely absent, so that the placenta is firmly attached to the uterus.
- Placenta increta: Here the trophoblast invasion may extend far into the myometrium.
- Placenta percreta: Here the extravillous trophoblasts invade through the entire wall of the uterus and may even infiltrate the bladder wall (in the case of an anteriorly located placenta).

In all three situations, placental delivery after birth is often difficult or impossible

and is associated with massive maternal bleeding. Reasons for the increasing number of placenta accreta/increta/percreta cases have now been identified. With an increase in cesarean sections in previous births (Silver et al. 2006) as well as after successful endoscopic surgery for Asherman syndrome (Fernandez et al. 2006), the risk of deeper trophoblast invasion also increases. This may indicate that the myometrium arrests trophoblast invasion. In areas where the musculature is absent, e.g. due to scar tissue, this blockade is absent, resulting in deeper trophoblast invasion (Sect. 8.1).

- **Insufficient Invasion**

On the other hand, insufficient invasion and thus anchorage of the placenta to the uterine wall can lead to premature detachment of the placenta, either before birth or during labor.

An almost complete absence of the endoarterial trophoblast characterizes the placental bed of miscarriages that occur late in the first trimester and have chromosomally normal fetuses (Ball et al. 2006a). Interestingly, no irregularities in trophoblast invasion were found in very early miscarriages (Ball et al. 2006b).

If the endoarterial trophoblast fails to occlude all arteries connected to the placenta during the first trimester, local influx of oxygen-rich maternal blood into the intervillous space may occur too early. This has dramatic consequences for the corresponding area of the placenta, such as a marked reduction in trophoblast proliferation and decreased villous angiogenesis. This leads to disruption of placental development resulting in miscarriage in severe cases. Mild cases may result in severe fetal growth restriction (FGR) of the fetus with or without preeclampsia (Kadyrov et al. 2003; Jauniaux et al. 2003b). The premature influx of maternal blood into the placenta can be clinically demonstrated by transvaginal ultrasound (Jauniaux et al. 2003b).

These clinically relevant deviations from the norm highlight the importance of understanding extravillous trophoblast invasion in detail.

1.5.6 Maternal Perfusion of the Placenta

The perfusion of the placenta with maternal blood differs fundamentally between the first trimester and the two later thirds of pregnancy. In the first trimester of pregnancy, maternal perfusion takes place with an ultrafiltrate of blood without blood cells (blood plasma), but with secretion products of the uterine glands (histiotrophic nutrition of the embryo). During the other two thirds of pregnancy, the placenta is perfused with maternal blood (hemotrophic nutrition of the fetus).

In the First Trimester

Already at the time of the early villous phase, the first superficial capillaries of the decidua are eroded by the invasion of the trophoblast, which then flush a few blood cells into the developing intervillous space. However, these few blood cells do not set up a maternal circulation through the placenta at this time of pregnancy.

Later during the first trimester, the spiral arteries are opened towards the placenta by the endoarterial trophoblast but are directly occluded before an influx of maternal blood into the placenta can occur (Burton et al. 1999). Thus, no maternal blood cells are found in the placenta during the first trimester, only a maternal plasma stream. This apparent lack of maternal blood cells in the intervillous space has been demonstrated by transcervical endoscopic examination of the intervillous space (Schaaps and Hustin 1988) as well as by Doppler ultrasound of the placenta (Jauniaux et al. 1991).

However, there is a permanent flow of fluid through the intervillous space already

in the first trimester. The plugs of the endoarterial trophoblasts do not allow blood cells to pass, but they do allow the passage of plasma, which then enters the intervillous space as a clear fluid. To this the secretion products of the uterine glands must be added, which are admixed with the plasma. This fluid, rich in lipids, nutrients and growth factors, is called uterine milk and is crucial for the nutrition and thus development of the placenta and embryo.

Due to the absence of blood cells, only the oxygen physically dissolved in the plasma reaches the placenta during the first trimester. Thus, a physiologically low partial pressure of oxygen of <20 mmHg is found in the placenta until about the 11th week of gestation (Jauniaux et al. 2000). The occlusion of the arteries already connected to the placenta and thus the low oxygen concentrations during embryonic development can be explained as follows (Huppertz et al. 2009):

- The reduction of oxygen leads to the reduction of free radicals and thus protects the rapidly growing embryo from teratogenesis during this critical phase of tissue and organ development (Jauniaux et al. 2003a).
- Mammalian cells, especially embryonic cells, grow faster under low oxygen conditions than at high oxygen concentrations. Embryonic development is characterized by rapid growth and cell division. Thus, low oxygen concentrations at this time are ideal for creating an environment in which a high cell division rate needs to be be maintained.
- The connection between the placental and embryonic blood vessels via the developing umbilical cord is not fully established before the 7th week of pregnancy. Thus, full perfusion of the placenta with high oxygen concentrations from the maternal side would not reach the embryo at all before then.
- Perfusion with maternal plasma without maternal blood cells could protect the early syncytiotrophoblast from coming into direct contact with circulating maternal immune cells.

In the Second and Third Trimester

Only towards the end of the first trimester, the intra-arterial plugs of endoarterial trophoblasts dissolve and maternal blood with blood cells flows into the intervillous space of the placenta. This results in a marked increase in the intraplacental oxygen content. Thus, partial pressures of oxygen of up to 60 mmHg are present in the placenta at the beginning of the second trimester (Jauniaux et al. 2000). Studies in primates and humans have shown that by about the 11th week of gestation, the influx of maternal blood into the placenta is detectable (Roberts et al. 2016). By term, the partial pressure of oxygen in the placenta falls slightly, but still remains at around 40–45 mmHg (Soothill et al. 1986).

It is important to note that these oxygen partial pressures in the placenta with very low values in the first trimester, the strong increase at the beginning of the second trimester and finally the slight decrease towards term must be described as physiological and normoxic. Thus, especially in the first trimester, it is not a matter of hypoxia, but of physiologically low but normal values of oxygen in the placenta.

In the third trimester, villous trees are found in the placenta, which have developed into large tree-like structures starting from the early trabeculae in the lacunar phase of placental development. It can be roughly estimated that each of these villous trees is surrounded by maternal blood from a central transformed spiral artery. At term there are between 40 to 100 of these arteries. The outflow of maternal blood from the intervillous space takes place via about 50-200 uteroplacental veins, which are the veins that have been connected to the placenta by the endovenous trophoblast. Their openings

are mostly found between the villous trees, so that there is a more or less directional blood flow from the incoming arteries via the villous trees to the venous openings. As a result of the lack of remodeling of the venous walls by the endovenous trophoblast, these vessels may not be involved in the regulation of intervillous blood flow.

1.5.7 Formation of the Chorion laeve (fetal membranes)

With the encasement of the embryo by the syncytiotrophoblast during early placentation, a spherical placenta is formed at this time. This remains intact for weeks and only changes its shape with the perfusion of the placenta with maternal blood at the beginning of the second trimester.

In the first trimester, the placenta is spherical but has a developmental gradient from the site of implantation (the embryonic pole of the placenta) to the side facing away from implantation (the abembryonic pole of the placenta) (Huppertz et al. 2009). This abembryonic pole is covered by a thin layer of decidual connective tissue (decidua capsularis) as well as the uterine epithelium, while the embryonic pole is oriented toward the uterine tissues. The earlier developed embryonic pole retains this developmental advantage throughout the first trimester.

This developmental advance results in more extravillous trophoblasts invading the decidua basalis from the cell columns on the embryonic side of the placenta, while this number is rather low at the abembryonic side of the decidua capsularis. Thus, at the embryonic side more spiral arteries are invaded, and the invaded arteries are more deeply invaded and blocked with a greater number of endoarterial trophoblasts.

The small number of endoarterial trophoblasts blocking the spiral arteries at the abembryonic side cannot withstand the pressure of the maternal blood for long. Thus, the first influx of maternal blood into this side of the placenta occurs well before the 11th week of gestation. The placental tissue in this area is unable to cope with the significant increase in oxygen and the associated oxidative stress. From about the 8th/9th week of gestation, the villous tissue in the area of the abembryonic pole begins to degenerate. Eventually, the villi are degraded and the intervillous space on this side of the placenta collapses.

Now the decidual connective tissue of the decidua capsularis comes into direct contact with the chorionic plate, and thus towards the end of the first trimester the fetal membranes, the chorion laeve, is formed (Jauniaux et al. 2003b).

Only at this later stage of the first trimester, the plugs in the spiral arteries on the embryonic side are dissolved, and towards the end of the first trimester maternal blood flows into this side of the placenta as well. Also at that time of pregnancy it can be shown that oxidative stress in the villous tissues is significantly increased (Jauniaux et al. 2003b). In the majority of cases, however, the placenta survives this threefold increase in oxygen and thus becomes a disc-shaped placenta, as it is also present at birth (placenta discoidalis).

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Immunology of the Fetomaternal Border

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Contents

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2

2.1 Background

The field of reproductive immunology has been founded in the 1950s by Sir Peter Medawar, who first raised the question of why the allogeneic fetus is not rejected by the maternal immune system (Medawar 1953). Current knowledge of the immunology of reproduction as well as the fetomaternal interface has been summarized in several international publications or special volumes in recent years, which are recommended for more intensive studies of the topic (Svensson-Arvelund et al. 2014; Arck and Hecher 2013; Szekeres-Bartho et al. 2015). The present chapter does not claim to give more than a rough impression of the topic.

2.2 Immunology of the Endometrium

Pregnancy is immunologically prepared even before it begins. The female immune system, especially in the reproductive tract, has to ensure that no immune reaction occurs to the allogeneic sperm and seminal plasma, but that simultaneously protection against germs remains active. The seminal plasma is immunoregulatory active (Filippini et al. 2001), and at the same time, the uterus is an immunologically highly active and complex organ. The endometrium, which renews itself cyclically, must thereby also regularly rebuild its immunological capacities, while permanently ensuring sufficient functional defense. The non-pregnant endometrium contains almost the entire spectrum of immune cells, but with changes adapted to the menstrual cycle (Kammerer et al. 2004).

The number of maternal leukocytes in the uterus increases at the end of the menstrual cycle, especially after the time of possible implantation of the blastocyst. The largest proportion of these cells are uterine natural killer cells (uNK cells), approx. 70% of the immune cells are uNK cells. If implantation takes place, their number further increases. 20–30% of the immune cells are macrophages and about 2% are dendritic cells (Kammerer et al. 2004). Less frequent are T and B cells as well as neutrophilic granulocytes and mast cells. Thus, uNK cells and macrophages form the two largest cell populations of endometrial leukocytes. The functions of the different immune cells are very diverse. uNK cells and macrophages regulate trophoblast cell invasion and are involved in remodeling spiral arteries, while T cells and dendritic cells provide adequate immune tolerance and immune response to uterine infections (Bulmer et al. 2010). Some studies have shown that shifts in the immunological balance in the endometrium, such as those caused by an increase in uterine NK cells, can negatively affect implantation and early placentation (Tuckerman et al. 2010) (Fig. 2.1). The possible effects are currently still controversially discussed (Mor 2008).

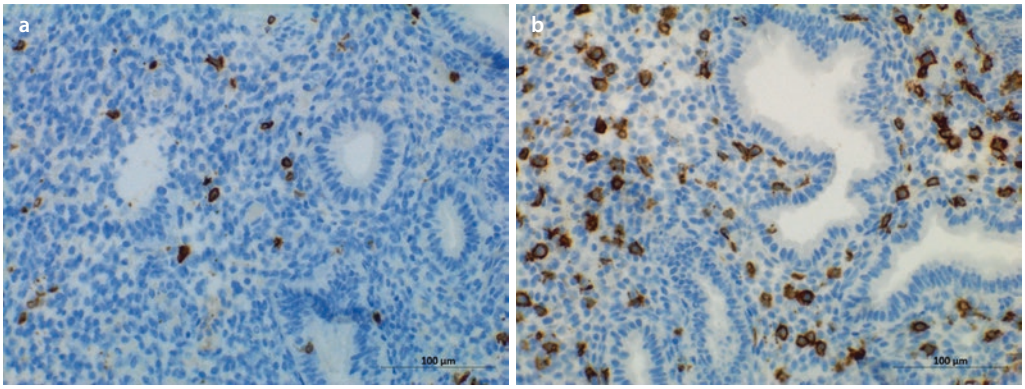

Fig. 2.1 **a**, **b** Uterine killer cells (uNK cells) labelled brown with anti-CD56 in the endometrium of the luteal phase (both on the 21st day of the cycle) (200× magnification). **a** Normal concentration; **b** Increased concentration

2.3 Immunology of Pregnancy

2.3.1 Problems

The immune system plays a special role during pregnancy, because on the one hand it must protect against infections, and on the other hand it must not develop a rejection reaction towards the embryo (Svensson-Arvelund et al. 2014). The embryo or fetus expresses 50% paternal genes that are foreign to the mother, so that a defensive reaction of the maternal immune system would be expected. Thus, it represents an intrauterine allograft that experiences first confrontations with the maternal immune system on its way through the fallopian tube.

During the first trimester, macrophages, dendritic cells and uNK cells accumulate in the decidua around the invading trophoblast cells. The latter release cytokines that interact with the immune cells of the decidua. Through Toll-like receptors, trophoblast cells can recognize bacteria, viruses, and pollutants, which further increase their cytokine secretion (Mor 2008). Thus, successful pregnancy and placental development depend on the function and interaction of trophoblast cells and maternal uterine immune cells (Trundley and Moffett 2004).

This interaction suggests an immune reaction against the fetus (Mor 2008), but it induces tolerance mechanisms, which imply that despite the increase in immune cells, a rejection of the fetus does not occur.

Although pregnancy is defined as an anti-inflammatory state, implantation of the blastocyst into the decidua resembles an inflammatory response with an increase in proinflammatory cytokines (e.g. TNF) and chemokines in the endometrium (Mor 2008; Redman et al. 1999). This may serve to control extensive trophoblast invasion early in pregnancy. The second immunological phase of pregnancy is characterized by rapid fetal growth and an anti-inflammatory state. The initially high concentration of uNK cells decreases, while the concentration of T lymphocytes increases. During the prenatal period, proinflammatory cytokines predominate and are involved in labor development, uterine contraction, delivery, and placental rejection (Mor and Cardenas 2010).

2.3.2 Fetomaternal Interfaces

Two of the obvious tasks of the placenta are to ensure a bidirectional exchange of substances between mother and child, but also to keep the two different individuals and in particular their blood circulations separate from each other. In doing so, two principally different immunologically important interfaces develop: the blood-trophoblast interface and the decidua-trophoblast interface (Fig. 2.2). At the blood-trophoblast interface, maternal blood bathes the syncytiotrophoblast-enveloped placental villi. At this site, most of the mutual exchange of substances takes place. Maternal blood contains high concentrations of humoral and cellular factors of the immune system, which would normally have to recognize the allogeneic villous surface as foreign and attack it, especially since the latter grows to several square meters during pregnancy. Using ex vivo perfusion of human placentas, we were able to show that immune cells are able to penetrate the syncytiotrophoblast barrier from the maternal circulation and migrate into fetal capillaries (Schamberger et al. 2013). The significance of this microchimerism is subject of current research.

The second interface, the one between invading trophoblast cells and decidua proves to be considerably more complicated. Here, fetal extravillous trophoblast cells infiltrate and invade maternal tissues, where the volume concentration of lymphocytes is

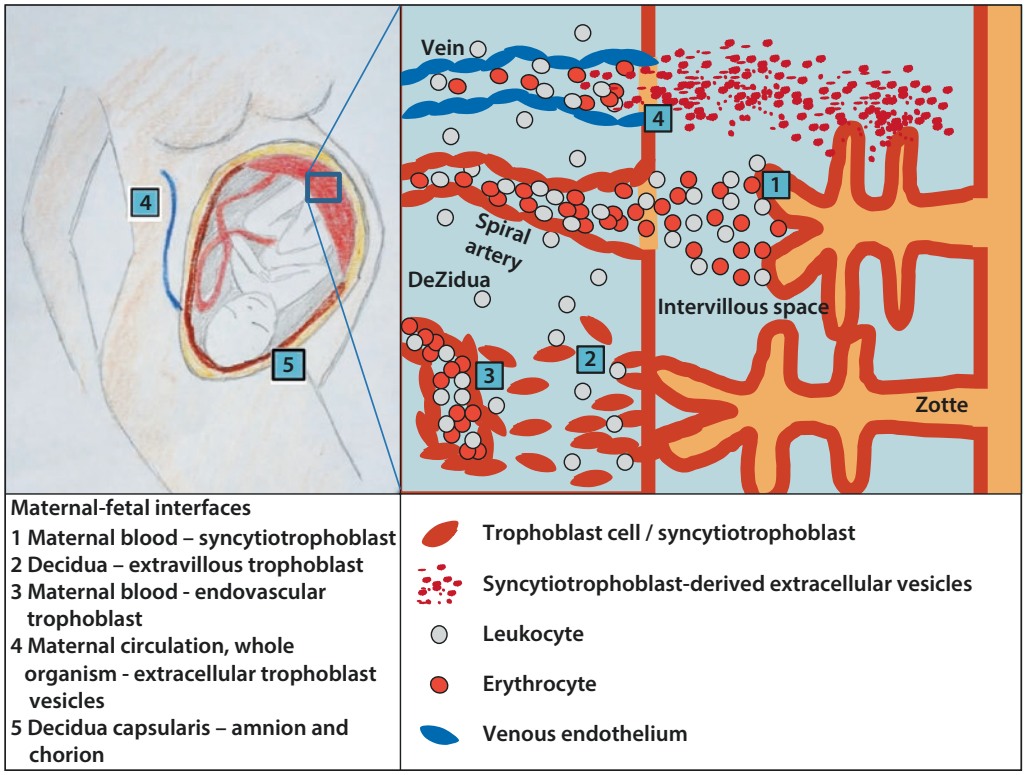

Fig. 2.2 Schematic representation of the five interfaces between mother and fetus at which cells or vesicles of fetal origin come into contact with maternal immune cells or their products

higher than that of blood, but with a different distribution of the various subpopulations: in the first place, uNK cells and cytotoxic $CD8^+$-T lymphocytes appear here, i.e. cells responsible for the elimination of foreign cells (Kammerer et al. 2004). However, the invading trophoblast cells escape or regulate these cytotoxic effects. Apparently, cytokines and growth factors of the activated maternal uNK cells paradoxically serve as stimulators for fetal trophoblast cells to migrate and proliferate (Faas and de Vos 2017). Eventually, trophoblast cells migrate in a purposeful, strand-like manner towards maternal blood vessels in the decidua, pushing between endothelial cells, displacing and replacing them. This process is regulated by immune cells, primarily uNK cells (Smith et al. 2009). The trophoblast cells that have replaced the endothelial cells are in turn in permanent contact with maternal blood, thus forming a third interface. Compared to the contact between blood lymphocytes and trophoblast cells, the contact between tissue-derived defense cells and trophoblast cells is of considerably longer duration, so that the quality of the mutual influence of these cells also differs.

Another interface exists between the fetal chorion and the maternal decidua capsularis, which fuse during pregnancy and press against the opposite decidua parietalis in the cavum uteri to form the fetal membranes. This chorion laeve has distinct immunoregulatory properties (Silini et al. 2017).

A fifth fetomaternal interface is represented by trophoblastic cells or particles in the maternal bloodstream or even in almost the entire body of the mother. Trophoblastic cells were detected in the lungs of deceased preeclamptic patients as early as in the 19th century (Schmorl 1893). Today, extracellular trophoblastic vesicles are in the focus of research on the influence of the placenta on the maternal organism (Foster et al. 2016).

2.3.3 Pregnancy: A Th2 Phenomenon

A crucial component for the non-rejection of the fetus is the immunological balance in the placenta with numerous anti-inflammatory and immunoregulatory factors. This is achieved by suppression of the T helper 1 (Th1) immune response in favor of the Th2 response. Therefore, pregnancy was first described as a Th2 phenomenon in 1993 (Wegmann et al. 1993). Th2 cytokines promote the maintenance of pregnancy, whereas an increase in Th1 cytokines can lead to abortion.

CD4-positive T helper cells can differentiate into Th1 and Th2 cells, which produce various cytokines. Th1 cytokines (tumor necrosis factor [TNF], interferon γ [IFN-γ], interleukins 2, 12, and 18 [IL-2, IL-12, IL-18], and others) activate macrophages and induce an inflammatory response via cellular immune defense. The Th1 immune response is also called cellular response because it leads to activation of NK cells and macrophages with the help of proinflammatory cytokines, such as IL-2, IFNγ, and TNF. Through these processes, intracellular parasites and tumor cells can be eliminated outside pregnancy. Thus, the Th1 response is very important for the defense against numerous infectious diseases. In addition, the pro-inflammatory cellular Th1 response is responsible for transplant rejection reactions and thus potentially dangerous for the allogeneic embryo or fetus. It must therefore be suppressed or regulated to protect the embryo/fetus. Th2 cytokines (IL-4, IL-5, IL-6, IL-9, IL-10 and IL-13) mediate a humoral immune response. The Th2 or humoral immune response stimulates B cells or plasma cells and leads to antibody production. Some Th-2 cytokines have implantation- and invasion-promoting effects on the embryo and trophoblast cells, respectively (Chaouat 2013).

Furthermore, immunosuppressive molecules produced locally by the placenta provide an immunoprotective milieu, including IL-10, prostaglandin E2 (PGE2) and transforming growth factor β (TGFβ). Decidua-derived PGE2 has been shown to block the activation of maternal T cells and NK cells (Moore and Persaud 2007).

2.3.4 Regulatory T Cells

Immune activity in the placenta is controlled by specialized regulatory T lymphocytes (Treg cells). They were already described as suppressor T cells in the 1970s, but were only characterized to a limited extent at that time. They suppress the activation of immune reactions and increase self-tolerance. They can be identified mainly by the expression of CD4, CD25 and Foxp3. Based on their Foxp3 expression, they were first detected in the non-pregnant endometrium in 2005 (Saito et al. 2005). They can be detected to be elevated in maternal blood as early as the early first trimester and accumulate in the decidua (Guerin et al. 2009). In several studies, reduced numbers or dysfunction of Treg cells have been associated with pregnancy complications such as miscarriage and preeclampsia (Steinborn et al. 2012).

2.3.5 γδ-T Cells

γδ-T cells represent a subpopulation of T cells. Although they comprise only a small proportion of the total population (1–10%), they contribute a crucial part to fetal tolerance (Mincheva-Nilsson 2003). The γδ-T lymphocytes have a special activation spectrum and are not limited to the classical human leukocyte antigens (HLA), as is the case with the αβ-T cells. They also recognize the non-classical HLA-G antigens (Barakonyi et al. 2002).

In the proinflammatory early pregnancy phase, (murine) γδ-T cells secrete, among others, TNF and IFN-γ. In the symbiotic later phase, on the other hand, they produce pregnancy-promoting molecules, such as TGF-β, IL-1β, IL-6 and IL-10 (Mincheva-Nilsson 2003). Similar to T helper cells, γδ-T cells also expose CTLA4 on their surface, which inhibits the activity of cytotoxic T cells (Mincheva-Nilsson 2003).

2.3.6 Natural Killer Cells

NK cells are derived from $CD34^+$ hematopoietic progenitor cells and, like B and T cells, belong to the lymphoid cell lineage (Caligiuri 2008). Immunophenotypically, NK cells are characterized by the expression of CD16, the Fcγ receptor III, CD56, an isoform of the neural cell adhesion molecule (NCAM), and by the absence of the T cell marker CD3 (Colucci et al. 2003).

In contrast to B and T cells, NK cells are able to identify certain virus-infected or malignant transformed cells by means of non-antigen-specific receptors and to eliminate them directly, without prior immunization or pre-activation. In addition, they modulate the immune response by secreting various cytokines and chemokines. These capabilities make NK cells a significant component of the nonspecific immune defense, an important mediator between innate and adaptive immune systems (Barakonyi et al. 2002).

Uterine NK Cells

NK cells are detectable in large numbers in the uterus during pregnancy. In the first trimester of pregnancy, about 40% of the decidual stromal cells are leukocytes. With a proportion of up to 70%, the majority of these are $CD56^{bright}$ $CD16^-$-NK cells. Although the number of decidual NK cells decreases in the course of pregnancy, they can still be detected in large numbers in the

decidua in the third trimester of pregnancy (Bulmer et al. 2010).

A striking phenomenon is the localization of decidual NK cells in close proximity to extravillous trophoblast cells and maternal spiral arteries. This position supports the assumption that decidual NK cells are activated by secretion of chemokines such as IL-8, IFN-γ-inducible protein-10 (IP10), or stromal cell-derived factor 1 (SDF-1), regulate the migration of extravillous trophoblast cells, and by releasing angiogenic growth factors such as placental growth factor (PGF) and vascular endothelial growth factor (VEGF), support the remodeling and new formation of maternal blood vessels. In addition, they presumably contribute to the maintenance of immune tolerance to invading extravillous trophoblast cells by inducing regulatory T cells (Vacca et al. 2013).

Although the functions of decidual NK cells as well as the underlying mechanisms have not been conclusively elucidated, their crucial role in pregnancy outcome is undisputed (Jabrane-Ferrat and Siewiera 2014; Seshadri and Sunkara 2014).

The origin of the decidual NK cells is controversially discussed. It is not clear whether they proliferate predominantly in tissue or are continuously recruited from blood. The chemokine receptor CXCR4 is expressed by $CD56^{bright}$ $CD16^{-}$ -NK cells in peripheral blood and binds the chemokine ligand CXCL12, which is detectable on extravillous trophoblast cells. Since extravillous trophoblast cells partially replace the endothelium of the maternal blood vessels in the decidua, they are in direct contact with maternal blood and can attract NK cells contained therein (Tao et al. 2015). Decidua and trophoblast cells produce other chemokines, such as CXCL10, CX3CL1 or CCL3, which elicit a chemotactic response of peripheral NK cells. In vivo, NK cell migration is likely regulated by multiple, sometimes redundant, chemokine signals. Within the decidua, local factors such as IL-11 and IL-15 influence NK cell differentiation and proliferation (Santoni et al. 2008; Carlino et al. 2012).

2.3.7 Uterine $CD14^{+}$ Cells

CD14-positive cells ($CD14^{+}$ cells) include monocytes, macrophages, Langerhans cells, neutrophil granulocytes and B cells. Monocytes arise from pluripotent myeloid stem cells of the bone marrow, which reach various organs and tissues and make up about 5–10% of blood leukocytes. They circulate in the blood for a few days before differentiating into macrophages or dendritic cells (Gordon and Taylor 2005). They can be identified immunohistochemically by their surface proteins CD14 and CD68. CD14 detects bacterial lipopolysaccharides, while CD68 detects lysosomal-associated proteins and is a marker for phagocytotic cells (Bulmer et al. 2010). Macrophages have immunoregulatory properties. They can have immunosuppressive effects and produce Th2 cytokines, e.g. IL-1, IL-6, IL-10, IL-15 and indoleamine-2,3-dioxygenase (IDO) (Heikkinen et al. 2003). They are activated by Th2 cytokines such as IL-4, IL-13 and anti-inflammatory cytokines such as IL-10 and produce prostaglandin E2 (PGE2), which in turn decreases lymphocyte function, lymphocyte proliferation and cytotoxic T cell development in vitro. They are also involved in innate and acquired immunity by presenting antigens via MHC II and releasing proinflammatory Th1 cytokines, such as TNF, IL-1ß and IL-12, and superoxides upon stimulation with bacteria (Singh et al. 2005). Decidual macrophages secrete acid phosphatase, nonspecific esterase, α1-anti protease and

α1-anti chymotrypsin, indicating their phagocytic properties. Accordingly, macrophages can protect against infection, but they are equally important for maintaining tolerance to the fetus (Heikkinen et al. 2003; Mor and Abrahams 2003). Macrophages support spiral artery remodeling via angiogenic factors (Svensson-Arvelund et al. 2014) and promote the invasiveness of extravillous trophoblast cells (Bulmer et al. 2010). They also influence the functions of their neighboring cells, such as trophoblast as well as glandular and vascular cells (Reister et al. 2001).

Following the Th1/Th2 system, macrophages are divided into proinflammatory M1 and immunoregulatory M2 macrophages (Gordon 2003; Gustafsson et al. 2008). M1 macrophages serve to protect against pathogens and induce IL-1, TNF-α and IL-12 release (Martinez et al. 2006). M2 macrophages support immune regulation and are regulated by IL-10 and M-CSF (monocyte colony-stimulating factor) (Svensson et al. 2011). Macrophages can switch between these two phenotypes depending on their environment such as the uterus (McIntire et al. 2008; Porcheray et al. 2005). In normal pregnancy, M2 macrophages are predominantly present in the uterus, while in preeclampsia patients, mostly M1 macrophages are present (Svensson-Arvelund et al. 2014). Trophoblast cells regulate monocyte migration and differentiation into $CD14^+/CD16^+$ macrophages (Aldo et al. 2014). Maternal macrophages are located in the decidua and intervillous space. Fetal macrophages are located in the stroma of the placental villi and are called Hofbauer cells. They show acid phosphatase and nonspecific esterase activity and thus the ability to undergo phagocytosis. They also form a second mobile barrier behind the syncytiotrophoblast layer (Reyes et al. 2017).

2.3.8 Trophoblastic Immunoregulatory Factors

HLA Class Ib

Extravillous trophoblast cells actively contribute to immune regulation through various mechanisms. One of the reasons why they are not eliminated by the maternal immune system is their expression of non-polymorphic MHC class I molecules of the type HLA-G, HLA-E and HLA-F, which are not recognized as foreign by cytotoxic T cells and reduce the cytotoxicity of NK cells (Persson et al. 2017). Binding to HLA-G actively inhibits maternal NK cells or stimulates them to produce anti-inflammatory cytokines. A soluble isotype of HLA-G causes NK cells to be inhibited even without direct contact with trophoblast cells (Poehlmann et al. 2006). Classical MHC class I molecules such as HLA-A or HLA-B are not expressed on trophoblast cells. If HLA-A molecules are transfected into trophoblast cells, their immunogenicity increases (Koc et al. 2003).

Indoleamine 2,3-dioxygenase (IDO)

IDO is an important enzyme for local immunosuppression in the placenta. It is secreted by the syncytiotrophoblast, uterine glands and antigen-presenting cells, among others. There is a concentration gradient between the fetal and maternal side (Blaschitz et al. 2011). Inducer of synthesis is mainly IFN-γ. IDO causes the degradation of tryptophan via a kynurenine cascade to acetoacetate and suppresses T-cell activity due to the resulting tryptophan deficiency, which is of fundamental importance for tolerance to the fetus (Mellor and Munn 2004).

However, immunosuppression is also caused by bioactive tryptophan derivatives. Thus, the deficiency of tryptophan results in both reversibly decreased T-cell prolifera-

tion and reversible inhibition of NK cells by kynurenine (Terness et al. 2007). Furthermore, IDO contributes to the local suppression of the T cell-mediated inflammatory response by inducing the formation of Treg cells (Chen et al. 2008).

2.3.9 Hormones

Numerous immunological regulatory processes are induced and controlled at the transcriptional level by the pregnancy hormones estradiol (E2), estriol (E3), and progesterone (P4), as well as by glucocorticoids (Robinson and Klein 2012).

Various immune cells, including lymphocytes, macrophages and dendritic cells, express progesterone receptors. In addition, P4 also binds to glucocorticoid receptors and thus has an anti-inflammatory effect (Jones et al. 2010). Progesterone induces the synthesis of leukemia inhibitory factor (LIF) and macrophage colony stimulating factor (M-CSF) (Arck et al. 2007) and is thus important for successful implantation and development of the embryo (Szekeres-Bartho et al. 2009).

Progesterone also influences the Th1/Th2 balance by causing the conversion of Th0 cells into Th2 cells, especially at the fetomaternal interface, and by reducing the secretion of Th1 cytokines (Piccinni et al. 2000; Saito 2000; Szekeres-Bartho and Wegmann 1996). Progesterone also leads to an increase in HLA-G production (Arck et al. 2007).

During pregnancy, progesterone receptors on lymphocytes are upregulated. When the hormone binds to the corresponding receptor, the progesterone-induced blocking factor (PIBF) is synthesized (Szekeres-Bartho et al. 2005). Similar to progesterone, it is an efficient inducer of immunosuppression. On the one hand, PIBF causes the shift towards Th2 cytokines (Szekeres-Bartho and Wegmann 1996) and on the other hand it inhibits arachidonic acid synthesis (Szekeres-Bartho et al. 2001). Arachidonic acid is the starting substance for the formation of many inflammatory mediators, such as leukotrienes or the prostaglandin E2.

In addition, PIBF inhibits the degranulation of NK cells and thus their cytotoxic effect on the fetus (Arck et al. 2007). PIBF not only plays an important immunoregulatory role in pregnancy, but is also used by tumors to reduce the immune response (Szekeres-Bartho and Polgar 2010; Ermisch and Markert 2011).

Human Chorionic Gonadotropin (hCG)

Another important immunoregulatory hormone is human chorionic gonadotropin (hCG). It is initially secreted by the blastocyst and later by the syncytiotrophoblast. Important effects of hCG include stimulation of progesterone production in the corpus luteum, activation of regulatory cells, increased angiogenesis through induction of VEGF expression, positive influence on trophoblast differentiation and migration, and increased release of pregnancy-promoting cytokines and growth factors such as M-CSF and LIF (Fournier 2016).

2.3.10 Other Selected Pregnancy-promoting Mechanisms

Fas/Fas Ligand

Fas and its ligand (FasL) are transmembrane proteins that belong to the TNF superfamily. Fas is also known as the "death receptor" and is found on activated lymphocytes, among others. Apoptosis is initiated as soon as a Fas/FasL interaction occurs. If a maternal lymphocyte with Fas binds to the FasL of the syncytiotrophoblast, it can per-

ish via a caspase signal chain by means of apoptosis (Crncic et al. 2005; Aluvihare et al. 2005).

Trophoblast cells co-express Fas and FasL. Therefore, they require a protective mechanism against autocrine-initiated destructive processes. This protective effect is provided by Bcl-2, an anti-apoptotic protein that is expressed by the trophoblast in addition to Fas and FasL (Aluvihare et al. 2005; Uckan et al. 1997).

Th1 cytokines, such as TNF, promote Fas expression in trophoblasts, while Th2 cytokines, such as IL-6 and IL-10, decrease trophoblast expression and sensitivity to Fas-mediated apoptosis (Makrigiannakis et al. 2008).

Galectin-1

Galectin-1 is a glycan-binding protein and also appears to play a significant role in fetal non-rejection. It is secreted mainly in the early gestational period by decidual NK cells, cytotrophoblast cells and the syncytiotrophoblast, among others (Kopcow et al. 2008). It causes a Th2 shift and an increase in progesterone and PIBF synthesis (Makrigiannakis et al. 2008). Galectin-1 also causes apoptosis of activated cytotoxic T cells (Kopcow et al. 2008).

Trophoblastic Extracellular Vesicles

Trophoblastic vesicles of >2 µm diameter were already described as being pinched off as syncytial knots from the syncytiotrophoblast at the end of the 19th century when they were discovered in the lungs of deceased pregnant women (Schmorl 1893). These fetal vesicles thus represent a further boundary or contact surface in the maternal organism.

Microvesicles or ectosomes (diameter: approx. 100–1000 nm), which are released from the apical syncytiotrophoblast membrane, and exosomes (diameter approx. 30–100 nm), which are released from intracellular multivesicular structures, serve to communicate between the fetus or placenta and the maternal organism, including the immune system. The vesicles are composed of proteins, microRNA, RNA and DNA (Chamley et al. 2014). Trophoblastic microvesicles express placental alkaline phosphatase (PLAP), among others, making them easily identifiable and distinguishable from vesicles of other origins (Gohner et al. 2015). In contrast, exosomes carry surface features from the cell interior of their cells of origin.

It has been shown in vitro that factors such as proteins or non-coding RNA produced in trophoblast cells can be transported via extracellular vesicles. When these vesicles are incubated with T lymphocytes, the trophoblast-derived RNA molecules are subsequently detectable in the lymphocytes and can influence the proliferation of target cells and thus the maternal immune system in pregnancy (Delorme-Axford et al. 2013; Ospina-Prieto et al. 2016). The concentration as well as the composition of trophoblastic extracellular vesicles are often altered in pregnancy pathologies. In preeclampsia, their concentration in the blood of pregnant women is significantly increased (VanWijk et al. 2002).

The fact that large amounts of extracellular vesicles enter the maternal circulation from the syncytiotrophoblast is unanimously accepted, but there are controversial results, especially about their composition in different diseases, largely stemming from the technical difficulties of isolation and analysis (Morales-Prieto et al. 2014).

2.4 Summary and Conclusion

In pregnancy, two allogeneic individuals live symbiotically together and their tissues, including the various immune cells, have immediate contact with each other. A num-

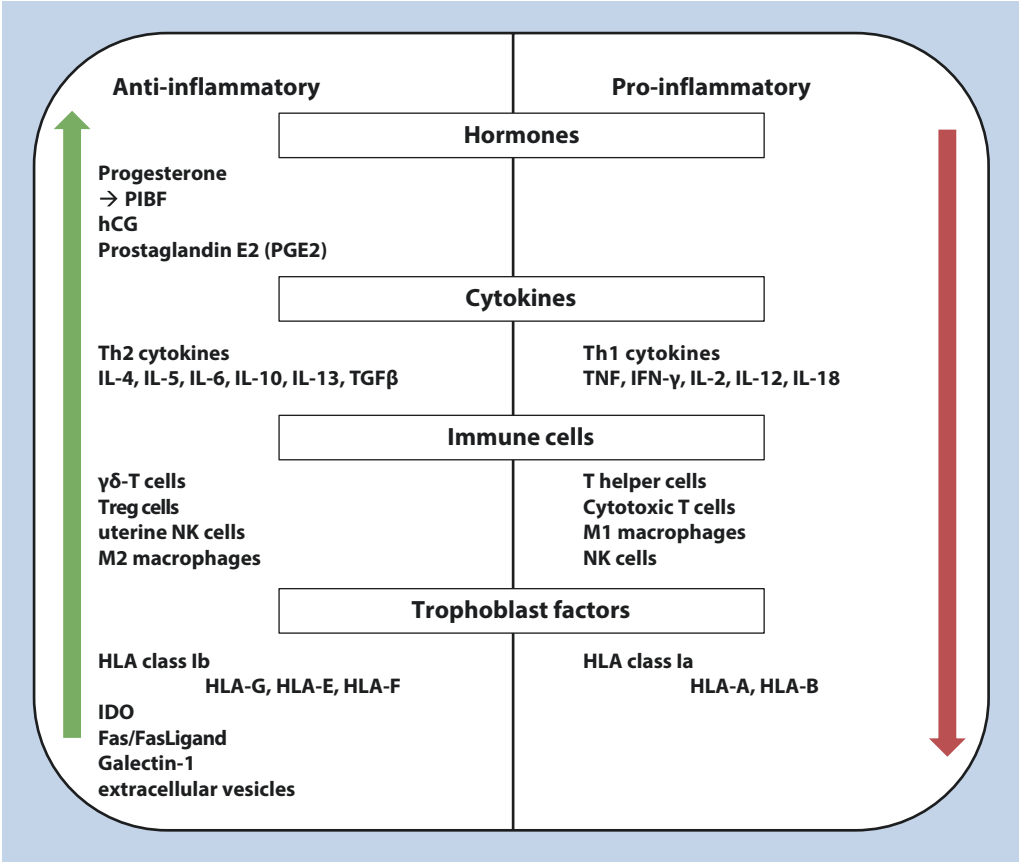

Fig. 2.3 Schematic overview of important immunoregulatory factors at the fetomaternal interface. A relative predominance of anti-inflammatory factors develops. Proinflammatory factors are partly completely absent or significantly reduced

ber of decidual and placental immunoregulatory cells and factors ensure the physiological course of pregnancy. In addition, the placenta releases factors into the maternal circulation that adjust her organism and immune system to pregnancy (Fig. 2.3). It has been widely described that disturbances in the immunological balance can negatively affect fertility and pregnancy and may lead to disorders or disease. Their better understanding will lead to better diagnostic techniques as well as to new therapeutic approaches.

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Placental Morphology

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3.1 Morphology of the Placenta

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3.1.1 Introduction

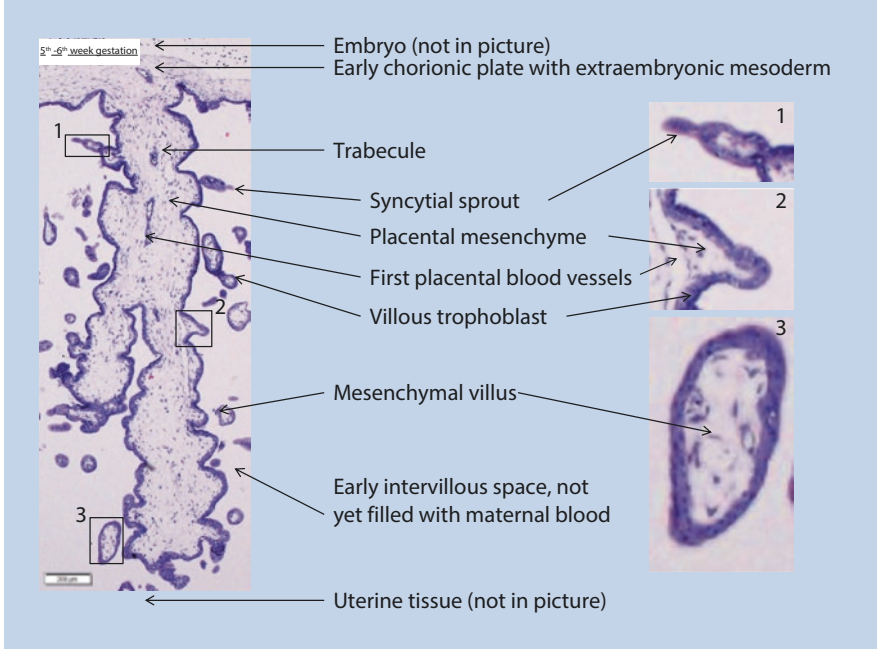
The placenta is formed with the development of the trophoblast even before the implantation of the blastocyst. During the embryonic period and subsequently during the fetal period of pregnancy, the placenta must perform its multiple and changing functions, while at the same time undergoing massive morphological changes.

This chapter section is devoted to the morphological features of the placenta over the duration of pregnancy and illustrates the features of the placenta in cross-sectional images during the course of a healthy pregnancy.

3.1.2 Villous Development

First villi develop around the 5th week of pregnancy (post menstruation, p. m.) starting from the large trabeculae of the syncytiotrophoblast (Chap. ▶ 1). These trabeculae still range from the early chorionic plate to the basal plate and thus reach the maternal tissues. ◘ Figure 3.1 shows such a trabecule at the 6th week of gestation with the first blood vessels in the placental mesenchyme and with syncytial sprouts leading to the development of the first mesenchymal villi. At this time, the intervillous space is still filled with blood plasma and glandular secretion products (uterine milk), maternal blood cells do not reach the placenta at this stage of pregnancy.

The driving structures in the formation of the villous tree are the sprouts of the syncytiotrophoblast (◘ Fig. 3.2a). These syn-



◘ **Fig. 3.1** Villous development in the 5th/6th week of pregnancy. Starting from the early chorionic plate, a large trabecule can be seen which projects towards the uterine tissue. The first villi develop from this trabecule by sprouting of the villous trophoblast

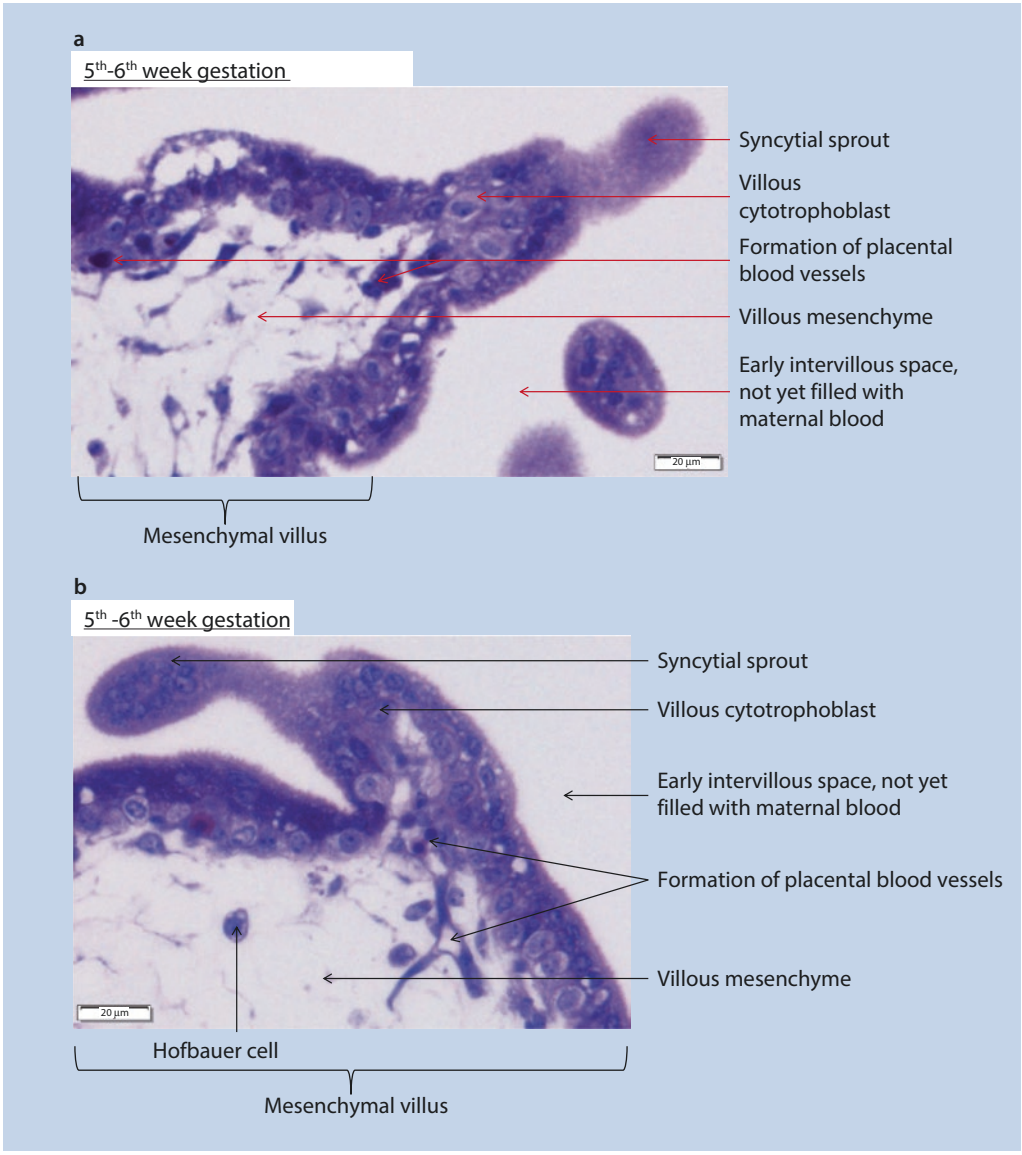


Fig. 3.2 **a**, **b** Villous development in the 5th/6th week of pregnancy. **a** A syncytial sprout indicates the development of a new villus. With the ingrowth of villous cytotrophoblasts, a primary villus is formed, which is then filled with connective tissue in its center (secondary villus). **b** The continued growth of a syncytial sprout indicates the direction for the formation of a new villus. This sectional image shows the ingrowth of blood vessels into the new villus, which thus becomes a tertiary villus

cytial sprouts bulge into the intervillous space on the outside of the syncytiotrophoblast and define the areas where new villi will be formed. Here, the underlying cytotrophoblast proliferates, giving rise to the first purely trophoblastic primary villi. Subsequently, villous mesenchyme pushes into these areas (secondary villus), followed by the development of blood vessels (tertiary villus). Thus, a complete tertiary villus

is present, which continues to grow and develops more syncytial sprouts (Fig. 3.2b), thus driving villous tree development.

The mesenchymal villi are the first type of villus to develop and are characterized by a dense mesenchymal stroma with many mesenchymal cells, a few, still developing blood vessels and a thick layer of villous trophoblast (Fig. 3.3). From these mesenchymal villi, the first additional type of villus develops from about the 8th week of pregnancy, the immature intermediate villus (Fig. 3.3). This type of villus is the precursor of the stem villi and reaches significantly larger diameters than the mesenchymal villi. Immature intermediate villi have a very specific characteristic: the stromal channels. In these villi, the channels run along the axis of the immature intermediate villi, are independent of blood vessels and are not lined by any epithelium or endothelium. Placental macrophages (Hofbauer cells) are frequently found in the stromal channels, which makes it easy to identify such cells in sectional images (Fig. 3.3).

In both types of villi, it is readily apparent that the villous trophoblast in the first trimester of pregnancy still consists of two complete layers: a complete layer of closely aligned villous cytotrophoblasts and a second layer consisting of the syncytiotrophoblast (Fig. 3.3).

Over the next few weeks of pregnancy, the composition of the types of villi does not change; this is primarily a time of further growth in the size of the placenta. Only starting at the 18th week of preg-

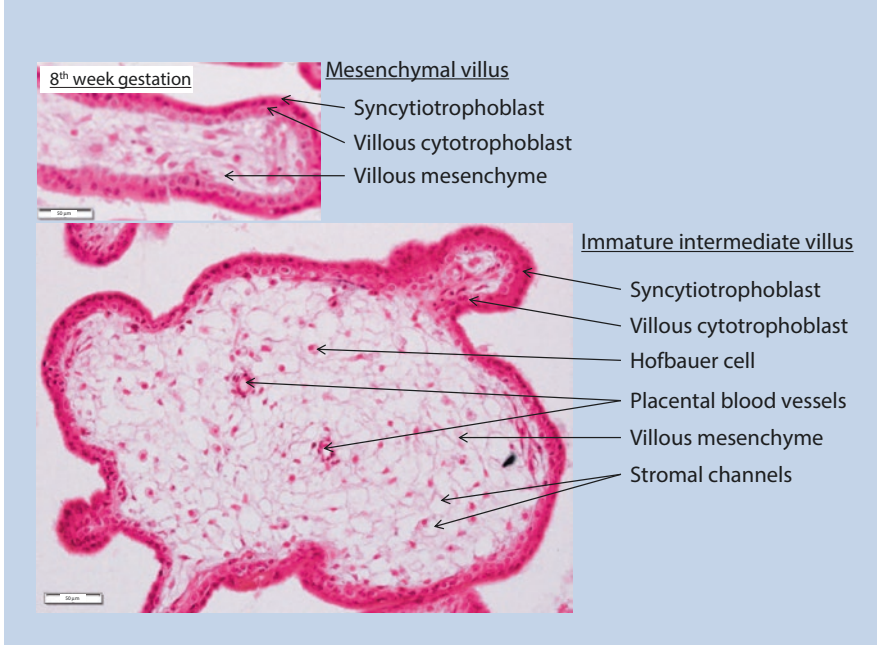


Fig. 3.3 Mesenchymal villus and immature intermediate villus at the 8th week of gestation. The images show a mesenchymal villus (*top*) and an immature intermediate villus (*bottom*). In addition to the typical histological features of these villi, the double layer of villous trophoblast (syncytiotrophoblast and cytotrophoblast) can be seen in both types of villi as well as the stromal channels, which are only found in the immature intermediate villi

nancy, the immature intermediate villi begin to differentiate into stem villi. This occurs from the inside to the outside and is clearly visible in sectional images. Figure 3.4 shows the differentiation of an immature intermediate villus into a stem villus. Due to a reorganization of the blood vessels, large central vessels develop in this villous type, around which extracellular matrix proteins (especially collagens) are now increasingly incorporated into the connective tissue. This process starts centrally around the vessels and continues outwards, so that at an advanced stage only marginal stromal channels are visible (Fig. 3.4).

After the stem villi have developed and are able to stabilize the villous trees, the remaining period of pregnancy is primarily dealing with increasing the exchange surface to allow sufficient nourishing of the fetus. This process is initiated with the development of the mature intermediate villi from about the 24th week of pregnancy. In histological sections, this type of villus is characterized by its elongated shape, its loose connective tissue and its many small blood vessel cross sections (Fig. 3.5).

At the end of the second trimester, it becomes obvious that the complete layer of villous cytotrophoblast is no longer present. Instead, isolated cytotrophoblasts

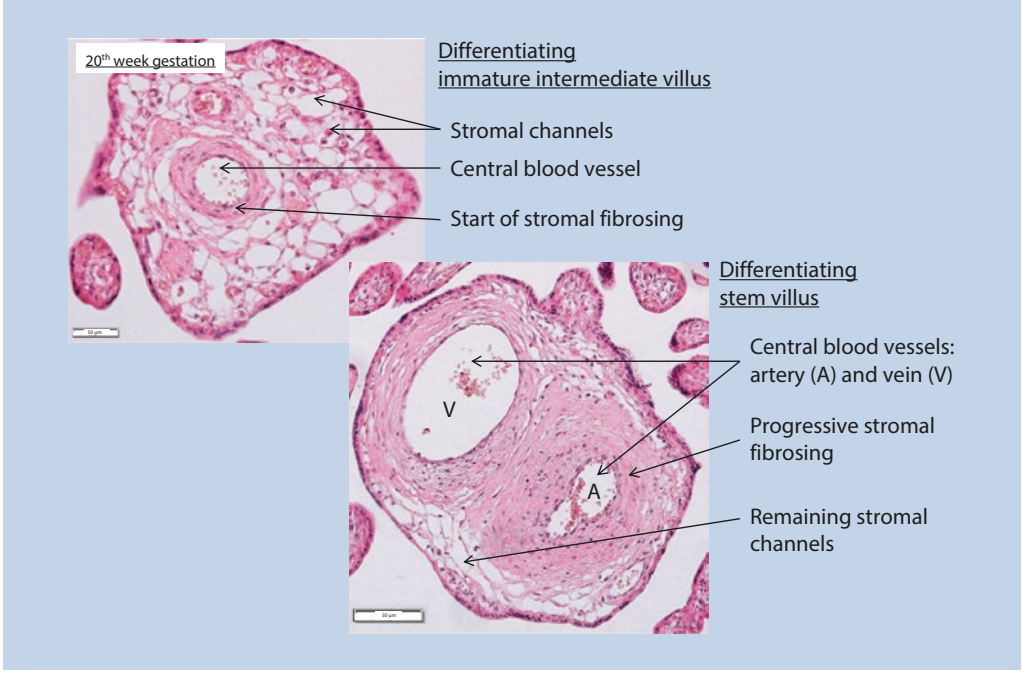


Fig. 3.4 Differentiation of the immature intermediate villi into stem villi at the 20th week of gestation. While the immature intermediate villus in Fig. 3.3 still shows the typical histological characteristics of this type of villus, it can be clearly seen that the immature intermediate villus in this figure (*top left*) is now differentiating towards a stem villus. The large vessel that develops in the center of the villus is striking. Further progressive differentiation leads to stem villi that are nearly complete (*bottom right*). Here the central vessels are surrounded by dense, fiber-rich connective tissue. Only at the margin, are there still remnants of loose connective tissue with stromal channels

are found between the syncytiotrophoblast and the villous stroma. Although the villous cytotrophoblast proliferates significantly during gestation, the growth pressure of the villous stroma is so strong that it pushes the layer of the mononucleated cells apart, leaving only isolated cytotrophoblasts in the sectional image (Fig. 3.5).

The mature intermediate villi form the basis for the development of the terminal villi as the type of villus that provides the largest area for exchange between mother and child at term. Terminal villi develop from about gestational week 27 and are characterized by a large number of capillaries as well as dilated capillaries (sinusoids) (Fig. 3.6). This type of villus will make up the largest proportion of all types of villi at the end of pregnancy to ensure adequate growth of the fetus.

Based on the above-mentioned processes of developmental changes in the types of villi throughout pregnancy, different villous sets result for the individual stages of development. These villous sets from the 15th to the 40th week of pregnancy are shown in Fig. 3.7.

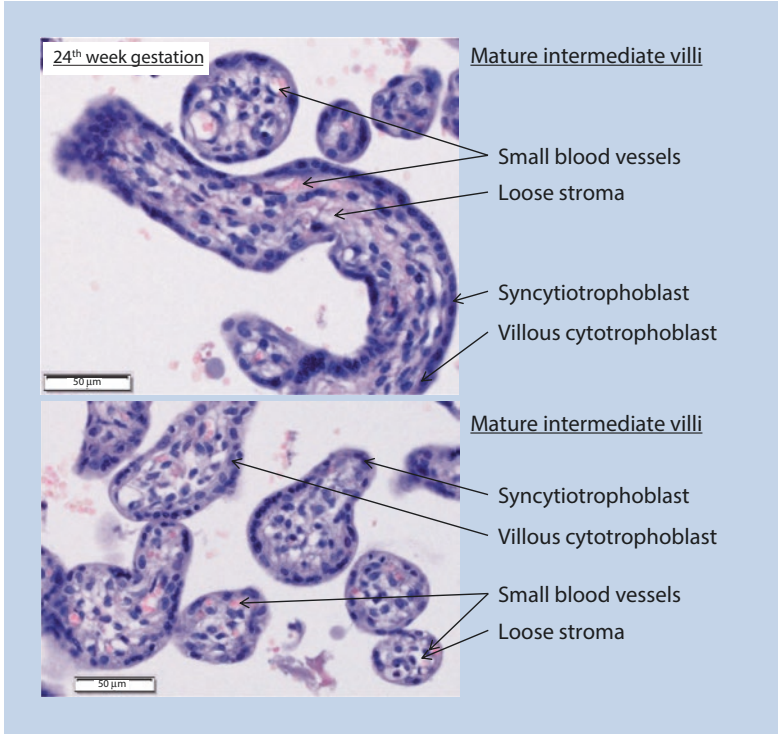


Fig. 3.5 Mature intermediate villi at the 24th week of pregnancy. At the beginning of the second half of pregnancy, the mature intermediate villi develop as a prerequisite for the development of terminal villi. This type of villus shows a distinct layer of the syncytiotrophoblast, under which only a few isolated cytotrophoblasts can be found

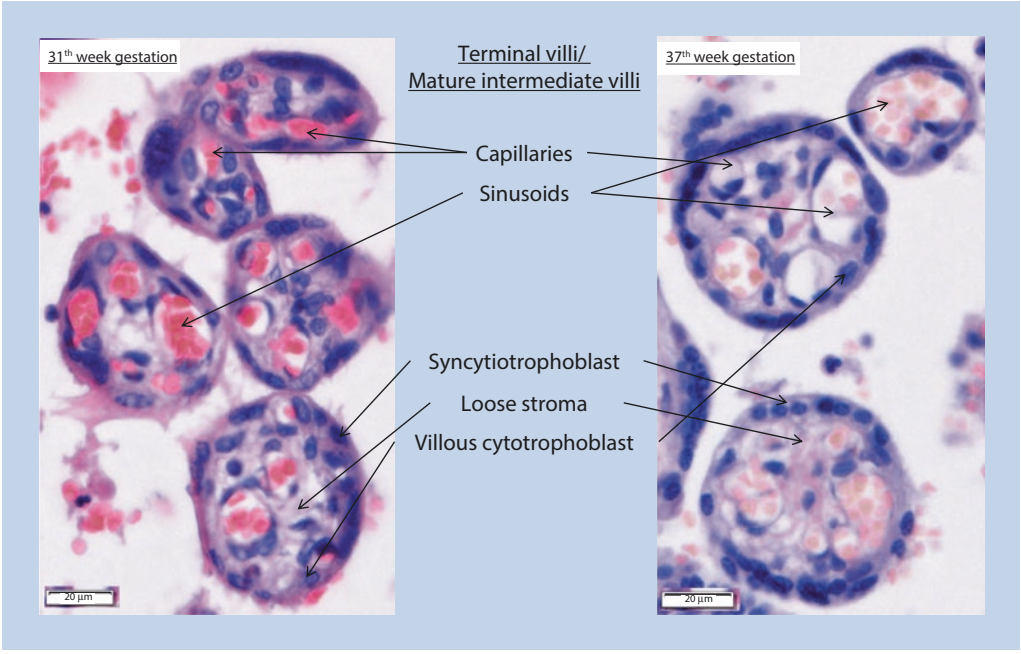


Fig. 3.6 Terminal villi and mature intermediate villi at the 31st and 37th week of gestation. These two types of villi are hardly distinguishable in diameter. Here, the presence of sinusoids must serve as a differential diagnostic feature, which distinguishes terminal villi from mature intermediate villi

3.1.3 Development of Cell Columns for Trophoblast Invasion

Trophoblast invasion by the extravillous trophoblast starts in the 5th week of pregnancy (p. m.) and continues throughout the entire duration of pregnancy. Initially, starting from a small placenta, a large number of invasive cells penetrate the maternal tissues of the uterus. As pregnancy progresses, the placenta expands and trophoblast cells can invade from a greater number of entry sites. The trophoblast cell columns as a source of invading cells thin out over the course of pregnancy, so that by term these cell columns are mostly completely depleted.

In the first trimester of pregnancy, multilayered cell columns are found that extend far beyond the boundary of the decidual tissue into the placenta, where they are surrounded by maternal blood plasma. This picture can be seen in the 5th–6th (Fig. 3.8a), as well as in the 8th week of pregnancy (Fig. 3.8b).

In the second trimester, the cell columns remain intact and a large number of extravillous trophoblasts find their way into the maternal tissue of the uterus (Fig. 3.9). The base of the anchoring villi widens, so that the cell columns also become wider and allow a better hold for anchoring the placenta to the uterine wall.

In the last trimester of pregnancy many cell columns are used up. Now the anchoring villi are firmly anchored to the basal plate, while only remnants of cell columns are visible (Fig. 3.10). Through the secre-

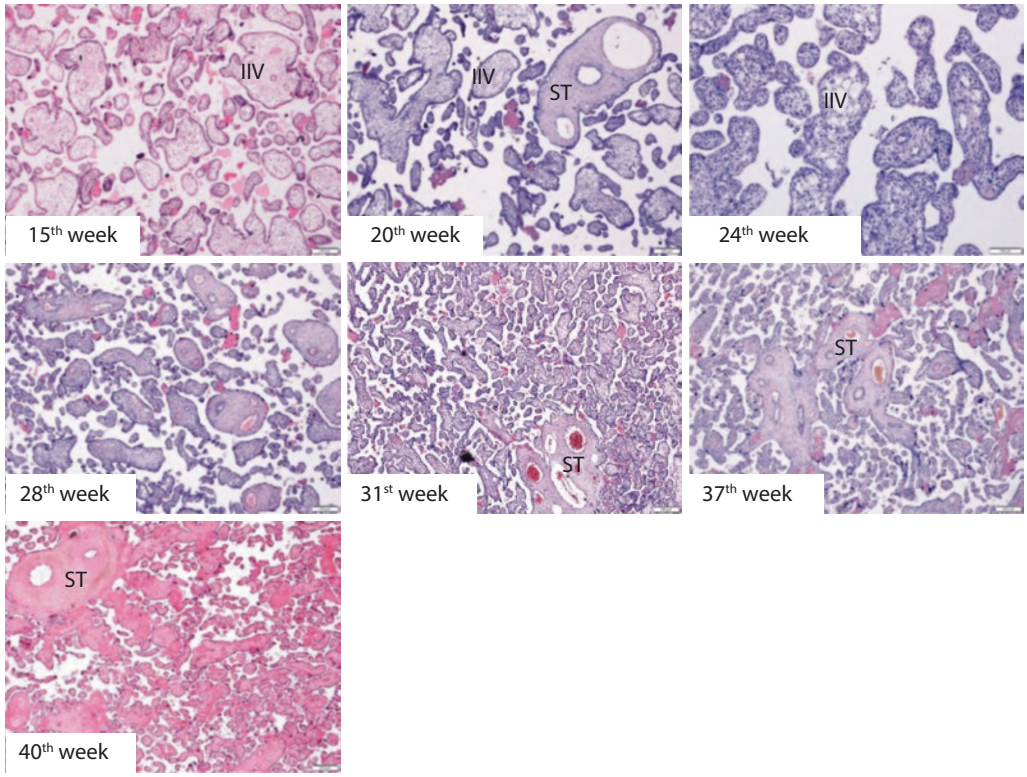
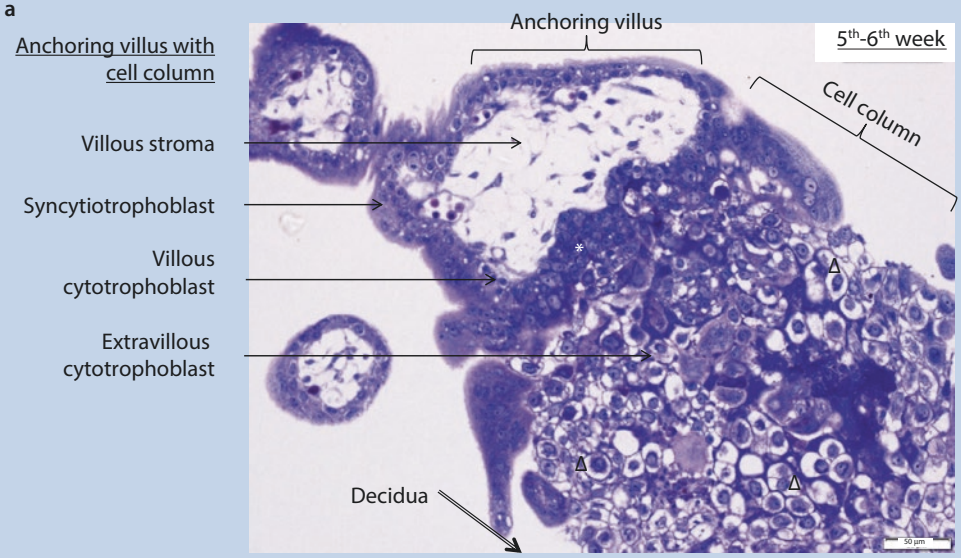


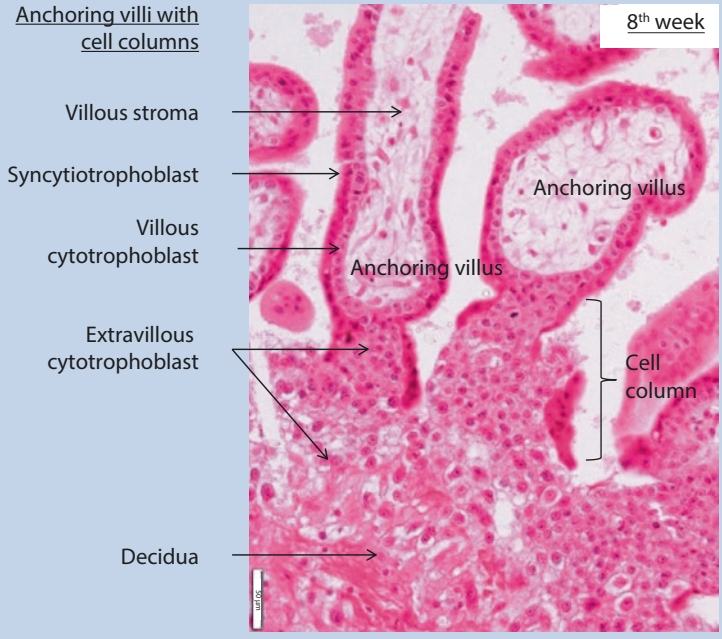
Fig. 3.7 Overview images of placentas between the 15th and 40th week of pregnancy. Over the course of pregnancy, it becomes obvious that the villi become smaller in caliber, but at the same time increase in number. This leads to a significant increase in surface area, which can be used for nutrient and gas exchange. It can also be seen that the large immature intermediate villi are very prominent in the first half of gestation, but are hardly found with the emergence of the mature intermediate villi. *IIV* immature intermediate villus; *SV* stem villus

Fig. 3.8 **a**, **b** Cell columns in the first trimester. In the basal region of an anchoring villus the trophoblastic cell columns are found, from which the extravillous trophoblasts invade the uterine tissues of the mother. In the proximal portion of the cell columns (**a**, *asterisk*) are the cells that are still actively proliferating. Proliferative pressure pushes subsequent generations of daughter cells toward the uterus. These cells then separate from each other and start their invasion into the decidua (**b**). The cells in the area of proliferation are clearly distinguishable morphologically from their daughter cells. While the proliferative cells are still small with dense and clearly stainable cytoplasm, the post-proliferative daughter cells are much larger and store glycogen, which is washed out during embedding. Hence, these cells appear pale and empty in a section (**a**, *arrowheads*)

a


Anchoring villus with cell column
Villous stroma
Syncytiotrophoblast
Villous cytotrophoblast
Extravillous cytotrophoblast
Decidua
Anchoring villus
5th-6th week
Cell column
50 µm

b


Anchoring villi with cell columns
Villous stroma
Syncytiotrophoblast
Villous cytotrophoblast
Extravillous cytotrophoblast
Decidua
8th week
Anchoring villus
Anchoring villus
Cell column
50 µm

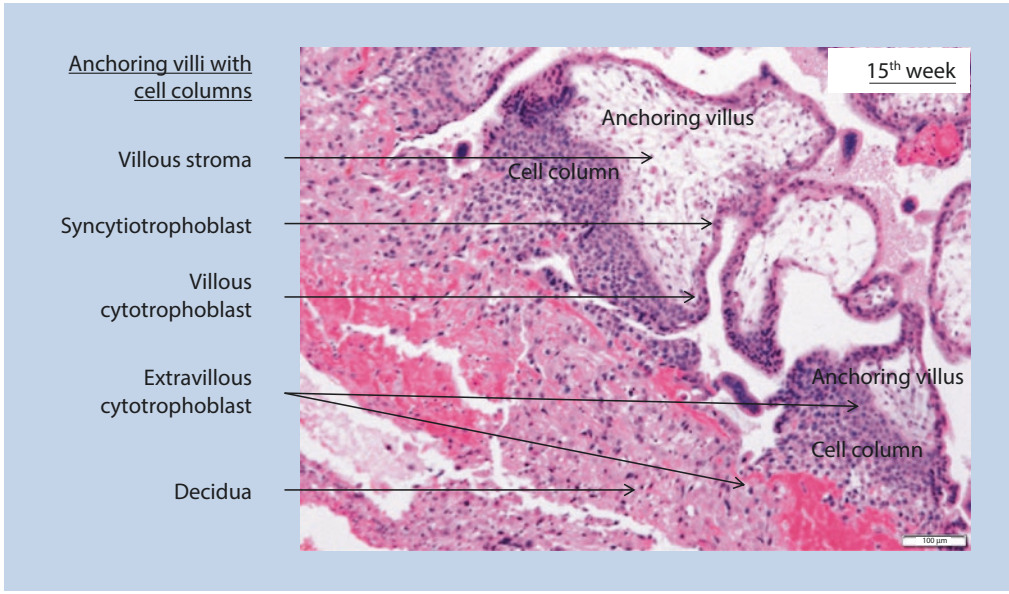


Fig. 3.9 Cell columns in the second trimester. In the second trimester the cell columns flatten and are no longer as high as in the first trimester. However, large amounts of extravillous trophoblasts continue to be formed, which invade the uterine wall and open uterine luminal structures

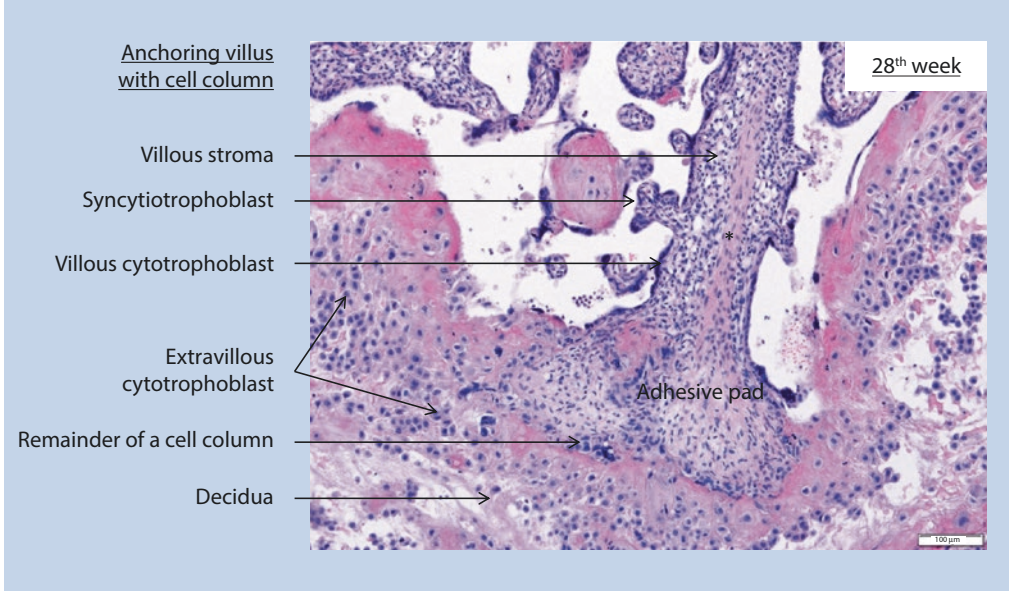


Fig. 3.10 Cell column in the third trimester. Cell columns are still found even in the third trimester. However, they can no longer perform their function as a source for the production of the supply of extravillous trophoblasts, as the proliferative part of the cell columns has been used up. The anchoring villi now extend into the decidua, are fibrosing in their center (*asterisk*) and are surrounded on their basal side by extravillous trophoblasts that have secreted large amounts of matrix-type fibrinoid. This allows the villous portion of the placenta to be well fixed to the basal plate

tion of matrix-type fibrinoid, the extravillous trophoblasts further solidify the area of the basal plate.

3.1.4 Structures on the Villous Surface

Especially in the mature placenta, different structures on the villous surface are often used to differentiate pathological from normal pregnancies (Fig. 3.11). On the one hand, there are protrusions of the syncytiotrophoblast such as the syncytial sprouts for villous development or the syncytial knots for the release of apoptotic nuclei. On the other hand, there are also changes in the three-dimensional structure of the villous trees, which result in changes in the sectional images and can be erroneously interpreted as protrusions. Thus, these structures are not always correctly assigned.

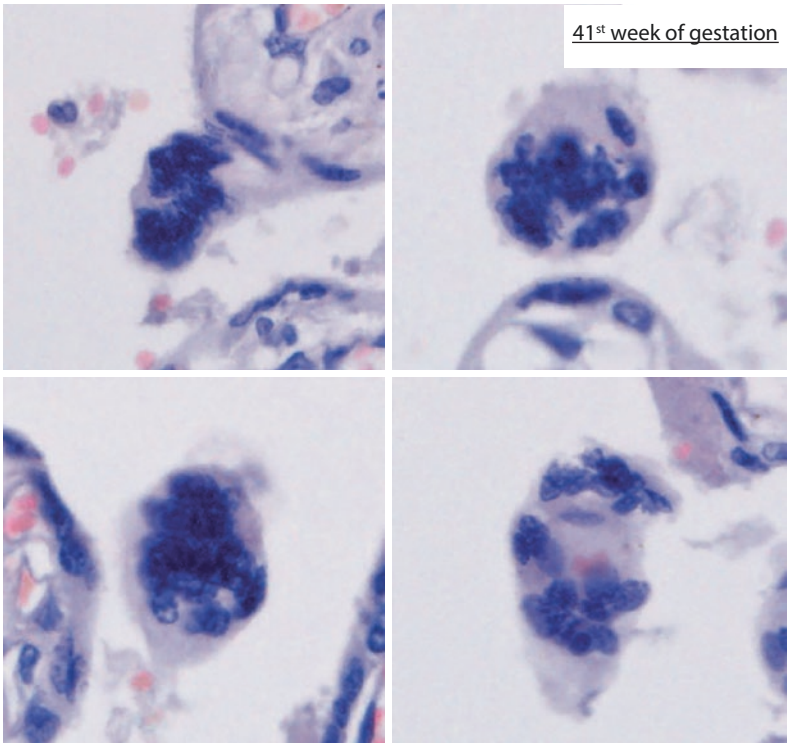


Fig. 3.11 Structures on the villous surface at the 41st week of pregnancy. In a mature placenta, corpuscular structures can be visualized on the villous surface. These are readily used to differentiate pathologic from normal pregnancies without distinguishing between these structures. Typically, at least three types of these structures can be distinguished: Outgrowths of the syncytiotrophoblast for villous development (syncytial sprouts), outgrowths of the syncytiotrophoblast for the release of apoptotic material (syncytial knots), and flat sections through the villous trees, which are difficult to distinguish from outgrowths. Since this is a placenta from the 41st week of gestation, there are two possibilities for the structures shown: syncytial knots or flat sections through the syncytiotrophoblast. Only a three-dimensional reconstruction of serial sections can clearly assign the true nature of such corpuscular structures

3.2 Histopathology of the Placenta for Gynecologists

Thomas Stallmach

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3.2.1 Introduction

The placenta is not comparable to any other organ. As it grows and develops its final architecture (maturation), it must perform a range of functions that will later be distributed among several organs. However, it only fulfils the requirements for nine months, and in some ways you can see it doing so. A tissue sample of the body organs of a young healthy person is usually immaculate under the microscope, while tissues from a term placenta without any complications always show read off pathological changes under the microscope. This makes the histopathological examination of a placenta a special task, in which the quantity of a change often plays a greater role than its quality. Thus, perivillous fibrin deposits in small quantity are apparently physiological, to be understood as the most favorable variant for repair of tissue defects in view of the remaining life of the organ. Above a certain amount of deposits and in the presence of further changes, however, the diagnosis of a maternal circulatory disorder arises, as is frequently observed in the context of preeclampsia. Unlike in the case of bodily organs, every examination of the placenta raises the question of whether a morphological finding is significant at all. If so, two aspects arise:

1. Were the findings so pronounced that a supply restriction or threat existed intrauterine or peripartum? This can be read off from the condition of the newborn if the nutritive function of the placenta was restricted, but is difficult to assess if the oxygen supply to the child was restricted.
2. Does the finding point to an underlying condition in the mother or child that may still be significant postpartum?

With largely perfected obstetrics, the vast majority of pregnant women will give birth to a healthy child, freed at this moment from the latent threat of a not always perfect short-lived multifunctional organ. In the following, placental pathology is presented arranged according to gestational age and disease groups, with a sentence at the end on the usefulness or necessity of postpartum examination of the organ appended to each group.

3.2.2 First Trimester (Abortion)

Human pregnancies are characterized by a relatively high rate of spontaneous pregnancy losses; this amounts to approx. 10% overall. After an abortion, the risk of a further abortion increases to 15%, and thereafter to 25%. The expelled tissue is usually sent for pathological examination to exclude malignant transformation of the trophoblastic tissue and to obtain an indication of the cause of the abortion.

Abortion with and Without Developmental Aberration

Apart from the exclusion of malignant transformation, the morphological examination of abortion material by a pathologist actually permits the clarification of only one question: Is the morphology of the placenta, in particular of the chorionic villi, so abnormal that a primary defect of the embryo must be assumed with great probability as the cause of the abortion? In the only two-dimensional sectional picture, the root-like branches of the chorionic villi reveal a certain harmony in an undisturbed pregnancy. The stroma of the chorionic villi is uniformly loose, equipped with fetal

blood vessels. The trophoblastic cover appears predominantly bilayered with polar broadenings interpreted as growth zones (Fig. 3.12a).

If the pregnancy is disturbed from the embryonic side, all three parameters may be disturbed. The architecture of the villous branching is irregular, the stroma is poor of cells and very loose, often in combination with villi whose stroma appears compacted, the trophoblast is partly atrophic, partly irregularly proliferating (Fig. 3.12b). Some of the changes may be pretended by regressive changes, which are almost the rule in the setting of a "missed abortion." If there is clear pathology in at least two of the three criteria (architecture, stroma, trophoblast), the diagnosis is "developmental aberration of chorionic villi". From this, a "primary embryonic cause of the abortion" can be deduced, which—as analyses of tissue from abortion material have shown—is usually based on a chromosomal aberration. The tissue of the placental basal plate and decidua often shows a band-like inflammation, which has set in as a result of the death of the embryo/fetus to demark the tissue.

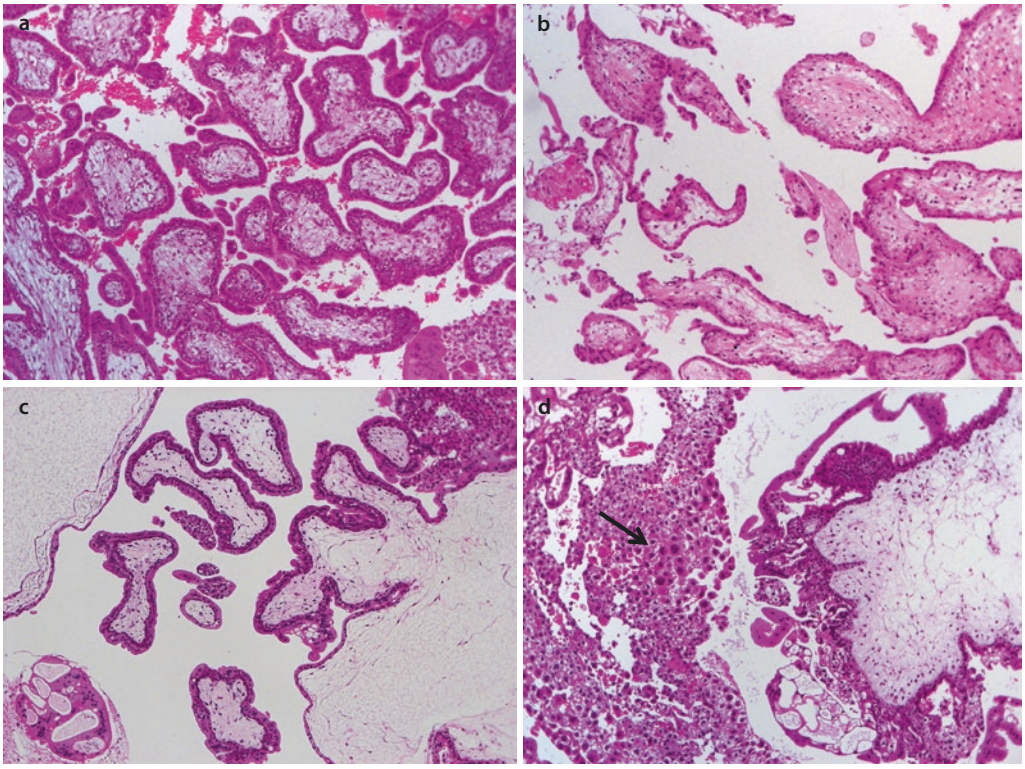


Fig. 3.12 **a** Regular morphology of the chorionic villi in the first trimester: regular branching pattern, regular nature of the villous stroma and two-layered trophoblast. **b** Aberration of the chorionic villi: irregular branching pattern, loose and dense stroma in alternation, atrophic trophoblast sections and trophoblast proliferation patterns. **c** Partial hydatidiform mole: Two morphologically distinct populations of chorionic villi, (I) nearly regular small villous caliber, (II) villous vesicle (fluid accumulation without stroma) with trophoblast proliferation, usually not covering the whole circumference of the villi and not showing severe atypia. **d** Complete hydatidiform mole: only vesicularly transformed chorionic villi (no normal villous population) with abundant trophoblast proliferation that often captures the entire circumference of the villi and shows distinct atypia (*arrow*). (H&E, 100×)

If a developmental aberration cannot be determined morphologically with the necessary certainty, the diagnosis can only state the absence of this finding. In some of these cases, necrotic chorionic villi surrounded by fibrin and hemorrhages of different ages are seen in the decidua, which is occasionally summarized as an "embryonic bed disorder". It remains completely open whether this is a primarily anatomical, hormonal or immunological problem, by which a subsequent pregnancy could also be affected, or a statistical event in the incipient remodeling of the maternal circulation in the region of the placenta.

Question About Hydatidiform Mole

Partial and complete hydatidiform moles also fall under the term "developmental aberration". However, the morphological picture is so typical that the chromosomal aberration behind it can be inferred immediately. In the case of a complete hydatidiform mole, this results in a specific risk profile. In the case of partial and complete hydatidiform mole, the term is derived from the vesicular enlargement of the chorionic villi.

Strictly to be distinguished from this is the "blighted ovum", in which an empty amniotic sac is seen as a vesicle. The absence of an embryo in a blighted ovum" is almost always an expression of a "developmental aberration" and will be accompanied by the above-mentioned morphological criteria of the chorionic villi. Etiologically, this is due to a multitude of spontaneously occurring chromosomal aberrations, but not to the specific chromosomal findings of hydatidiform moles.

Etiologically, the partial hydatidiform mole is based on a triploidy, i.e. in each cell of the embryo there is a triple set of chromosomes (karyotype 69 XXX, 69 XXY or 69 XYY). If two sets of chromosomes originate from the mother, the result is a dystrophic pregnancy with very small embryos/fetuses displaying characteristic anomalies. If two sets of chromosomes are of paternal origin, the result is a partial mole. An embryo is not formed in this case. Morphologically, chorionic villi fall into two distinct populations: largely inconspicuous chorionic villi contrast with vesicularly distended chorionic villi with irregular trophoblast. This gives rise to proliferation sites that are not polar (see above) but grow circumferentially in several directions without, however, showing any appreciable atypia (◘ Fig. 3.12c).

The complete mole is etiologically based on a zygote with two paternal chromosome sets without maternal chromosomal material. Again, no embryo is formed. All chorionic villi are vesicularly degenerated, do not show any blood vessels and are circumferentially covered by an exuberant trophoblastic proliferation within which atypical nuclei can be found (◘ Fig. 3.12d). The risk of a complete hydatidiform mole to progress into a choriocarcinoma is variably reported; both the frequency of complete moles and the risk of their malignant transforamtion appear to depend on ethnic factors. With an average risk of 1:50, a longer period of contraception is recommended to be able to detect the development of choriocarcinoma at an early stage by means of regular checks of βhCG.

Answers and Evidence from Examination of Abortion Material

The suspicion of a hydatidiform mole arises at the latest from the 10th week of pregnancy (ultrasound). In the case of abortions obtained earlier, the diagnosis of a hydatidiform mole can occasionally be made without any previous clinical suspicion. At the time of removal of a hydatidiform mole, it is unlikely that a pre-existing choriocarcinoma is present. Only complete hydatidiform moles, but not partial moles,

require a prolonged period of βhCG monitoring to rule out a choriocarcinoma. The risk of aberration after a partial mole is not different from the basal risk of aberration of trophoblastic tissue inherent in all pregnancies, this is reported to be 1:25,000. Thus, while the detection of specific findings (hydatidiform mole, congenital storage diseases already manifesting in the trophoblast) are rarities, the morphological evaluation of any abortion should include the statement: "developmental aberration present/not present/no definite statement possible at this point".

Face to face with the patient, the findings can be interpreted as follows. In the majority of cases, the abnormality refers to a chromosomal aberration (usually trisomy) in the embryo. In the vast majority of cases, such an aberration has occurred by chance during the development of the oocyte at the time of maturation. It is not necessary to know which aberrations are present. What is decisive is that the maternal organism (here the choice of words is a little delicate) has recognized the problem and the pregnancy has been terminated. It is not a negative omen for further pregnancies. This interpretation is not entirely without limitation. If the pregnant woman or her partner (!) is the carrier of a balanced chromosomal translocation, there is a systematic risk. In this case, one third of all further pregnancies will again be miscarriages. Of the delivered, (phenotypically) healthy children, half will also have the translocation. However, the statistically expected frequency of a pregnancy in which one of the partners is a translocation carrier is so low that this alone is not an indication for chromosomal testing of the parents after an abortion with a developmental aberration. In the case of abortions without evidence of a developmental aberration, the counselling situation seems less clear, since the cause cannot be clearly stated as being on the side of the embryo.

3.2.3 Second Trimester (Hydrops Fetalis, Infection and Inflammation)

Hydrops of Fetus and Placenta

Subcutaneous edema and effusions in the body cavities are called hydrops fetalis. In most cases the placenta is also hydropic and consequently pale and enlarged. The cause is a heterogeneous spectrum of diseases, led by various forms of fetal anemia. Hydrops may also be a consequence of congenital heart and lung malformations or an accompanying phenomenon of congenital tumors. In about 15% of cases there is a chromosomal aberration (e.g. monosomy X). Up to 30% of all cases remain unexplained.

Immunological Hydrops in Blood Group Incompatibility

Maternal antibodies directed against cells of fetal erythropoiesis destroy these cells and lead to fetal anemia. Prior to the recognition of the correlations and the development of effective therapy and prophylaxis (from 1969 onwards anti-D prophylaxis), these were mostly antibodies against the rhesus factor with prior sensitization of the mother, often through a previous pregnancy or abortion. In migrating populations that were previously poorly provided with medical care, the rhesus problem can now be increasingly observed again. In addition, sensitivities to other blood group antigens can rarely cause the same clinical picture. Hydrops of the placenta leads to pale and fragile tissue. This and the increase in size of the organ can occur rapidly analogous to the rapid development (but also possibility of improvement) of pulmonary edema in adults. The reason lies in the intrauterine circulatory situation. Both heart chambers work in parallel, the lung lies in a shunt. If increased circulatory work (due to anemia) leads to cardiac enlargement, muscular

insufficiency or even insufficiency of the AV-valves, this leaves the fetal lungs largely unaffected, but leads to congestion and development of edema in the systemic circulation. Microscopically, the edema of the chorionic villi is also striking, in addition to an increased number of Hofbauer cells. Numerous nucleated erythrocytes are found in the fetal blood vessels—otherwise hardly detectable in the second trimester (Fig. 3.13a). If the condition persists for longer, the chorionic villi also appear more immature with regard to their trophoblast covering compared to the gestational age.

Hydrops as A Result of Infection (Fifth Disease, Erythema infectiosum)

The destruction of cells of fetal erythropoiesis can also be the work of a virus. Parvovirus B19 is the causative agent of the generally little-known fifth disease. About 50% of women of childbearing age are immune. In the context of small epidemics,

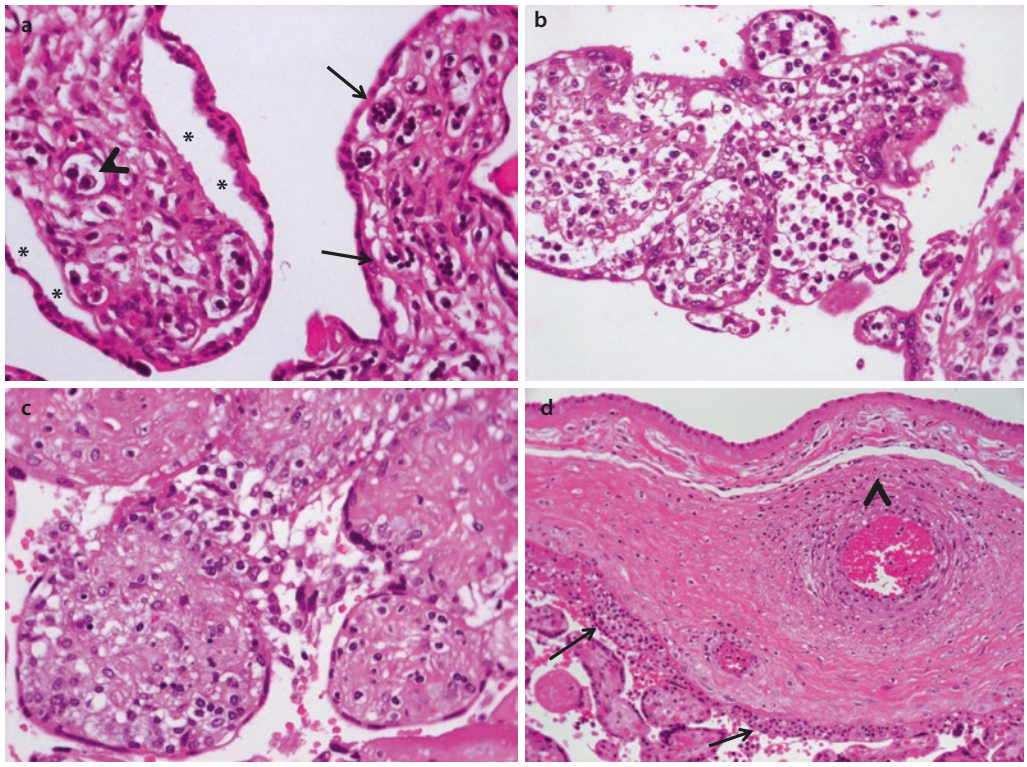


Fig. 3.13 **a** Hydrops in infection by parvovirus B19: The chorionic villi show increased fluid retention (= edema), which becomes obvious by lifted trophoblastic epithelium (*) in histological sections. In the fetal blood vessels there is a large number of nucleated erythrocytes, which have passed from the sites of fetal hematopoiesis into the circulation (*arrows*). This finding also arises as a consequence of destruction of erythrocytes in rhesus incompatibility. In addition, large erythropoietic cells with virus-typical nuclear inclusions are seen in the fetal circulation (*arrowhead*). **b** Acute villitis in maternal sepsis: Dense infiltrate of neutrophilic granulocytes within the chorionic villi. In a silver stain Campylobacter jejuni could be visualized. **c** Focal non-specific villitis in premature birth in the 30th week of gestation without evidence of infection: small foci of fibrin-clotted chorionic villi with loose mononuclear infiltrate. **a–c** H&E, 400×. **d** Early stage chorioamnionitis: maternal neutrophilic granulocytes as a dense fringe in the intervillous space below the chorionic plate (*arrows*). Fetal inflammatory cells have emerged from fetal blood vessels in the chorionic plate close to the amnion (*arrowhead*). (H&E, 200×)

seroconversion occurs in 3–7% of pregnancies. Symptoms occur in only 30% of infected pregnant women (transient anemia and skin rash); of the fetuses, only a small proportion becomes transplacentally infected. Within the fetal organism, apoptosis is induced by the virus in nucleated precursor cells of erythropoiesis; the resulting fetal anemia leads to hydrops due to circulatory mechanisms as in hydrops of immunological origin (◘ Fig. 3.13a). Since the virus is apparently capable of inducing fetal anemia for only a short time, it does not damage any other organs, and soon becomes undetectable. A rapid and marked improvement of the hydrops can be obtained by transfusion of blood to the fetus.

Placentitis

Placentitis (synonym "villitis") describes the morphological picture of inflammation in and around chorionic villi. Acute placentitis (◘ Fig. 3.13b) is caused by a group of viral and bacterial pathogens which spread hematogenously to the placental villi during maternal infection. Entry into the fetal tissues leads to inflammation, which can result in typical defects or malformations (e.g. rubella virus, varicella-zoster virus, cytomegalovirus, listeria and Treponema pallidum) or, via fulminant sepsis, to the death of the fetus (e.g. streptococci, staphylococci, campylobacter). If the morphological examination of the placenta reveals an inflammation of the chorionic villi without evidence of pathogens and usually without a clearly defined clinical picture in the fetus, this is referred to as chronic placentitis. The majority of these cases do not receive satisfactory etiological clarification.

(Acute) Inflammation of the Chorionic Villi with Evidence of Pathogens

- **Rubella virus (German measles):** An initial infection of the mother during pregnancy also leads to viremia in the fetus. The growth of fetal cells is temporarily slowed down by the infection. In the context of an inflammatory tissue reaction, malformations occur. The placenta shows a necrotizing inflammation in the chorionic villi, typically involving the fetal vessels (endarteritis/endangiopathia obliterans).
- **Varicella zoster virus (chickenpox):** The rare initial infection of the mother during pregnancy (especially in the period 8-20 weeks of gestation) leads to viremia in about 2% of fetuses, in which nerve cells in particular are attacked. Tissue atrophy occurs in the supply area of the associated nerve tracts and even mutilation of the extremities. The placenta shows only focal inflammation with necrosis and cell changes, which are suspicious for the presence of a viral infection.
- **Cytomegalovirus (CMV):** Not only the initial infection of the pregnant woman, but also reactivation during pregnancy with existing maternal immunity can lead to severe fetal damage. In the case of fetal survival, this results in particular in central nervous defects. In cases of fetal death (30% of cases), abundant characteristically altered virus-infected cells (owl eye cells) are found in the tissues. In the associated placenta, the detection of owl eye cells is a rarity; usually only a (non-specific) lymphoplasmacytic inflammatory infiltrate is observed.
- **Campylobacter jejuni:** Campylobacter is ingested from infected food (milk, poultry meat) and leads to acute bacterial colitis in the pregnant woman, which rapidly resolves. Apparently due to the altered immune status during pregnancies, maternal sepsis may develop and pass hematogenously to the fetus. While fetal sepsis is always fatal with Campylobacter, maternal deaths are rare. The chorionic villi show villitis with neutro-

phil granulocytes (Fig. 3.13b), and special staining can be used to see coiled bacilla.

- **Listeria monocytogenes:** Listeria are ubiquitous, are ingested through infected food and rarely lead to sepsis. In case of altered immunity and high infectious dose, sepsis may result in mother and fetus. Abscessing inflammation occurs in fetal tissues, and fetal lethality is 50% with residual central nervous damage in survivors. The chorionic villi show micro-abscesses with histiocytic cells, often involving the fetal membranes (Section "Chorioamnionitis").
- **Toxoplasma gondii:** Toxoplasmosis is a protozoa-caused infectious disease that is widespread in animals. Infection during pregnancy through infected food or contact with acutely diseased domestic animals can lead to hematogenous transplacental infection of the fetus. Intrauterine growth restriction occurs, preterm birth with psychomotor retardation usually results, and fetal death is rare. The placenta shows no inflammatory infiltrates, but intracellular pseudocysts in the amniotic epithelium, in which the pathogen lies in the form of the so-called tachyzoites.

(Chronic) Inflammation of the Chorionic Villi Without Evidence of Pathogens

In 5–10% of the patients examined (frequency depends on the indication for examination of a placenta), areas of fibrin-clotted chorionic villi (so-called Gitterinfarcts) with infiltrates of lymphocytes and macrophages, occasionally also plasmacytoid cells and giant cells are found (Fig. 3.13c). Pathogen detection is not successful. Among the children born, there is a somewhat high incidence of premature births and "small-for-date children"; while there is no evidence of an infection. A placenta during CMV-infection may show such a relatively low morphological finding, especially without specific findings (owl eye cells; see above), that only after exclusion of such an infection in newborns the diagnosis of a focal unspecific villitis (VUE, "villitis of unknown etiology") can be definitely made. It is most likely to be the mitigation of immunological phenomena at the fetomaternal border or the reaction to a primary intervillous circulatory disturbance.

Chorioamnionitis (Amniotic Infection Syndrome)

Physiologically, the lower genital tract is heavily colonized with bacteria, while the amniotic fluid is sterile. If bacterial colonization of the amniotic fluid does occur, both the maternal and the fetal organism react. Maternal granulocytes first infiltrate the subchorial fibrin (stage I; Fig. 3.13d), then the covering tissue (stage II), and finally lead to necrosis of the amniotic epithelium (stage III). Fetal granulocytes are seen first within the blood vessels of the chorionic plate (stage I; Fig. 3.13d), then in the wall of the umbilical artery (stage II), finally followed by necrotizing inflammation of the umbilical stroma (stage III). The most frequent consequence of the developing chorioamnionitis is premature birth. Intrauterine physiologic aspiration of amniotic fluid brings abundant inflammatory cells (fetal and maternal granulocytes) into the immature fetal lung tissue, which may be formally termed "intrauterine pneumonia." Surprisingly, only a small proportion of preterm infants show signs of infection. The aspirated inflammatory infiltrate is clinically associated with a clustered occurrence of "wet lungs", usually antibiotic treatment is not necessary. However, neonatal sepsis is more frequent in the collective "preterm infants with chorioamnionitis" compared to an unselected collective of born infants.

Umbilical Cord Infection

An inflammatory infiltrate in the umbilical cord is usually part of the morphological picture of chorioamnionitis and correlates with its temporal course. Frequently, the fetal granulocytes are seen only in the wall of an umbilical artery and are thereby sectorially directed against the surface of the umbilical cord (stage II). Rarely, necrotizing inflammation in Wharton's jelly leads to necrosis (stage III of the fetal response) or, in the later stages, to calcifications that are already visible to the naked eye. This needs to be distinguished from a fungal infection (Candida), in which macroscopic yellowish spots can be seen on the surface of the umbilical cord, microscopically accompanied by granulocytes without reference to the umbilical cord vessels.

Answers and Evidence from Examination of Placentas in Preterm Delivery

The diseases treated here as disorders of the second trimester may also extend to the third trimester, and some of the entities that will be discussed there (e.g. preeclampsia) often already affect the second trimester. In view of the overall low number of births in the second trimester and the usually serious pathology involved, the recommendation could be made to subject every placenta from this period to a pathological-anatomical examination. With a strict focus on individual diagnosis, one can leave all placentas without examination as an exception to this rule if they originate from circumstances that have already been clearly diagnosed (e.g. fulminant infection with an identified pathogen). Placentas resulting from interruptions in this period, whether due to chromosomal aberration or prenatally identified malformations, also do not require examination. In the case of a deceased child without a clear diagnosis, the examination of the fetus and placenta is of primary importance. If examination of the fetus by autopsy is not permitted, some cases can be satisfactorily clarified by examination of the placenta. It should be noted, however, that in the case of diseases (e.g. CMV), which are rapidly apparent on microscopic examination of the tissues of deceased infants, only discrete focal findings may be obtained in the placenta.

3.2.4 Third Trimester (Circulatory and Maturation Disorders)

In the third trimester, the placenta must provide the fetus, which is in principle also viable outside the uterus, with the basis for a further 5- to 6-fold increase in body weight. This process can be restricted by disturbances of the blood circulation or the maturation of the chorionic villi. If the pathological process particularly affects fetal nutrition, intrauterine growth restriction occurs. If this is a warning sign, premature delivery can be considered with the help of other diagnostic aids (e.g. Doppler ultrasound of fetal and placental blood flow). If the pathological process affects fetal oxygenation in particular, no warning signs are seen. The sad consequence is sudden intrauterine fetal death, which is responsible for about 25% of perinatal mortality in regions of well-developed obstetric care. Individual children can be saved if reduced oxygenation (restricted placental reserve capacity) is revealed by an "accidentally" performed oxytocin stress test.

Maternal Circulatory Disorder

The fetal chorionic villi are circulated around by maternal blood. Regional arrest of maternal blood circulation in the intervillous space results in spatially limited necrosis of chorionic villi. Larger, approximately spherical necrotic areas result from ischemia of a fetal flow path unit (cotyledonary infarction; ◘ Fig. 3.14a); smaller ischemic districts, variable in shape, are termed

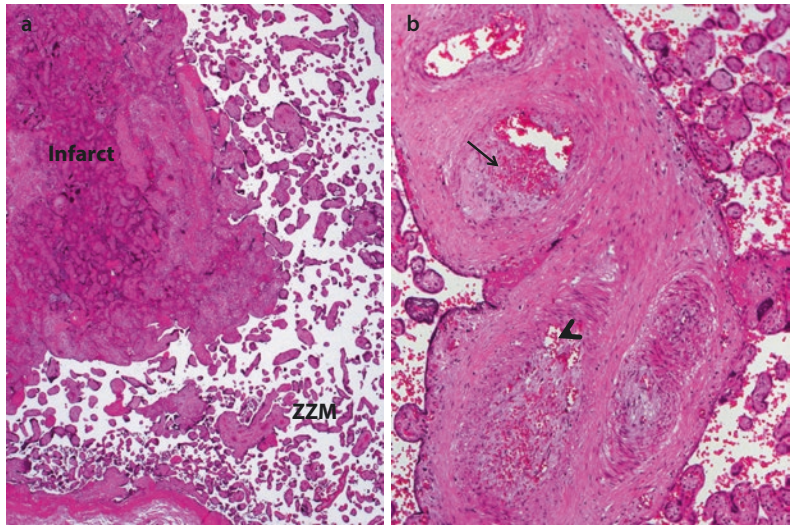


Fig. 3.14 **a** (Maternal) cotyledonary infarction: Large roundish limited district of intervillous fibrin and devitalized chorionic villi (infarction). The unaffected chorionic villi show preterm maturation to terminal villi. In the two-dimensional image of the chorionic villi many small calibers can be seen, while the growth compartment of the placenta in the form of the intermediate villi is reduced in quantity. The caliber gap between the stem villi and the terminal villi leads to the concept of intermediate villous deficiency (IVD). (H&E, 30×). **b** (Fetal) Endangiopathia obliterans: The blood vessels of a stem villus show an extensive lumen loss due to connective tissue proliferation sites, which are repeatedly interspersed by erythrocyte extravasations (*arrow*); in places recanalization is indicated (*arrowhead*). Neighboring chorionic villi show reduced to absent vascularization. (H&E, 150×)

Gitterinfarcts. In vivo, the maternal space between the chorionic villi (intervillous space) is of capillary width only. If the chorionic villi are forced apart by maternal blood clotted in the intervillous space, this is referred to as an intervillous thrombus.

Preeclampsia

Preeclampsia and HELLP syndrome ("hemolysis, elevated liver enzymes, low platelet counts") are clinically and laboratory-chemically defined disease states which, from an etiological point of view, conceal different disease processes. In severe cases, which become symptomatic as early as the second trimester and are associated with fetal growth restriction, examination of the placenta (typically in preterm birth, 25–28 weeks of gestation) reveals a defective remodeling of the maternal blood circulation to the intervillous space. Fetal-derived trophoblastic cell invasion (extravillous trophoblast) should have infiltrated the muscular walls of the maternal spiral arteries and remodeled them into wide muscle-free sections. If the activity of the extravillous trophoblast was too low, the inflow of maternal blood to the intervillous space is reduced with the consequences of decreased placental growth and the occurrence of infarcts (preferably Gitterinfarcts). Examination of the placental basal plate reveals a typical finding: maternal spiral arteries in which the intramural trophoblast cells are absent and which instead still have smooth muscle cells, interspersed in places with foam cells (acute atherosis).

Premature Placental Abruption

Premature placental abruption is a clinical diagnosis caused by a retroplacental hematoma. Its development may be favored by systemic (hypertension) and local factors (pathology of the basal plate in preeclamp-

sia). Examination of the placenta reveals an indentation of the basal plate with firmly adherent blood coagula. Further examination may reveal regional (small) infarcts and/or intervillous thrombi. These signs of a not only fresh intervillous circulatory disturbance are absent if the premature abruption was fulminant (e.g. due to abdominal trauma).

Fetal Circulatory Disorder

A placental circulatory disturbance on the fetal side is morphologically most likely to be recognized by the blood vessels in the section of the stem villi (endangiopathia obliterans). Unfortunately, the finding is not specific as to etiology, as it may occur in the setting of an infection (e.g., rubella infection) or as a reaction to an upstream flow obstruction (umbilical cord complication) and, to some extent, even as an irrelevant secondary finding following fetal death from another cause.

Endangiopathy Obliterans

If the placenta is macroscopically inconspicuous in intrauterine fetal death, the microscopic findings in the fetal vascular system often point the way. Fluctuating blood pressure and flow in the fetal circulation are a stimulus that induces endothelia to proliferate. Vascular cross-sections in the stem villi show restriction of the lumen by cell proliferation, sometimes with fragmented erythrocytes (◘ Fig. 3.14b); in later stages, connective tissue occlusions with multiple small capillary lumina (recanalization) are found. If this endangiopathy obliterans ("fetal thrombotic vasculopathy") is accompanied by inflammatory infiltrates, an infection must be sought (e.g. rubella, cytomegaly). The findings of an endangiopathy obliterans must be clearly recognizable and must be identified in numerous vessel cross sections. If the vessel wall is only edematous with dehiscence of the cells and presumed lumen restriction, this may be a phenomenon after an intrauterine fetal death, which has already occurred some time ago and which cannot be attributed to a vascular pathology.

Hereditary Thrombophilias

Fetuses that have received several thrombophilic hereditary factors from both parents (e.g. factor V Leiden, protein S deficiency) may already show intrauterine consequences of this multiple heterozygous condition within the coagulation cascade. The clear endangiopathy obliterans that develops early in this process is typically accompanied by a circulatory disturbance also in the maternal circulation (in the form of Gitterinfarcts). A coagulation-physiological clarification of the parents (today mostly in the form of genetic tests) is only indicated if signs of thrombophilic diseases can be elicited in the family history.

Umbilical Cord Complications

A true, tightly tightened knot in the umbilical cord is plausible as a cause of fetal circulatory disturbance. Obviously, also umbilical cord wrappings, in consequence of which a section of the umbilical cord is put under strong tension by fetal movements (between the base of the umbilical cord and the place of the wrapping) may lead to fluctuations of the blood flow. A clearly recognizable endangiopathy obliterans is then to be regarded as pathogenic; the finding of umbilical cord looping (in a dead child) is thereby recognized as pathogenetically relevant.

Maturation Disorder

Fetal blood flows through the umbilical cord and is distributed over the chorionic plate of the placenta, from where it finally circulates through the chorionic villi, which are densely packed in a "pot" (intervillous space) formed by the mother. The chorionic villi can be divided morphologically into three segments. The stem villi have blood vessels with muscular walls and a dense

stroma; they distribute blood to the intermediate villi. These are considered the growth compartment in the villous trees; blood vessels have a thin wall and the stroma is loose. The terminal villi are the actual functional compartment for exchange (Fig. 3.15a); it is here that the most impressive changes in morphology are found during pregnancy. The term maturation is used to describe qualitative and quantitative changes in the three types of villi during pregnancy. The term "maturation disorder" refers to clearly recognizable deviations from a morphological ideal type of villous differentiation (maturation) related to the respective gestational age. Pathogenetically, the maturation disorder may be an adaptive phenomenon (e.g. "intermediate villus deficiency" in the case

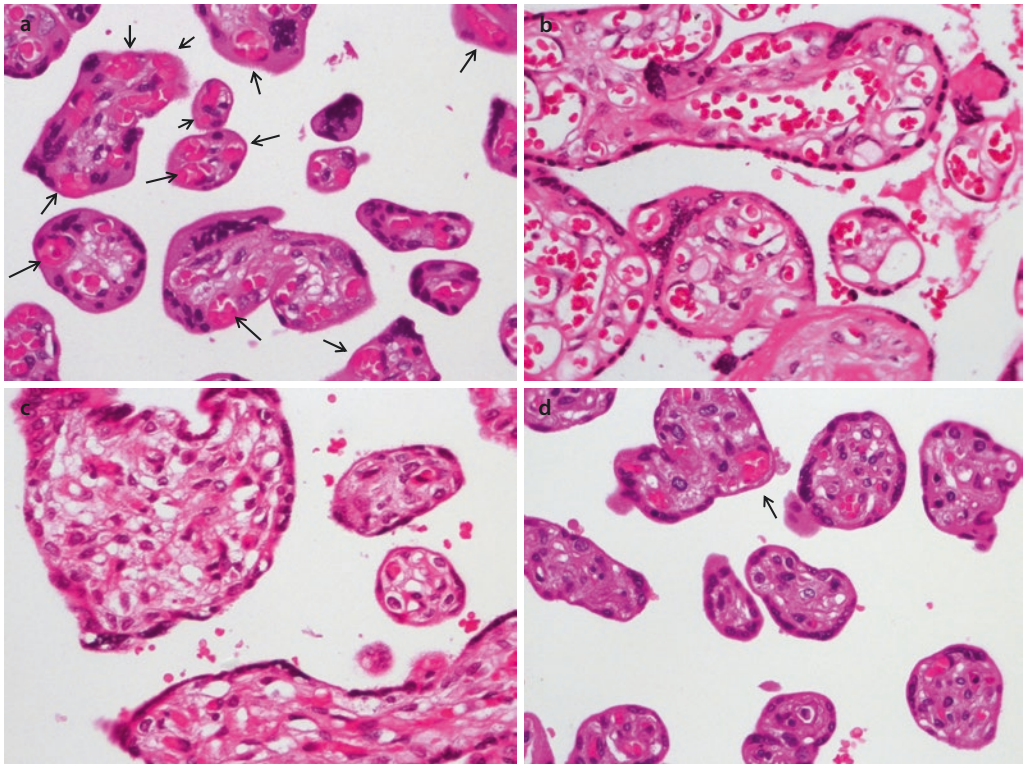


Fig. 3.15 **a** Normal villous maturity at term: From the 34th week at the latest, mature terminal villi dominate the picture quantitatively: The small villous cross-sections are occupied to approx. 30% by blood vessels, whose sinusoidally dilated lumina are only separated from the maternal intervillous space by a thin cytoplasmic membrane (syncytio-capillary membranes, *arrows*). **b** Chorangiosis type II: Morphological continuum of intermediate and terminal villi with enlarged cross-sections compared to the normal state and a significantly higher proportion of cross sectional area allotted to blood vessels. Sinusoidal and syncytio-capillary membranes are abundantly formed. **c** Retardation of villous maturation in diabetes: the morphological development of the placenta towards term stays behind "concordantly": both the branching pattern and the extent of vascularization of the chorionic villi appear to correspond to a much earlier gestational age. Macroscopically, the placenta is usually large, the sectional surface is too pale for the gestational age. **d** Terminal villus deficiency: the chorionic villi show a normal pattern of branching, in contrast, vascularization is retarded (discordant maturation retardation). There is a tendency to sinusoidal dilatation; however, syncytio-capillary membranes are much too rare (in the present image the example displays only one *arrow*, compare with **a**. Macroscopically normal-sized placenta, sectional surface is pale (H&E, 500×)

of a primary disorder in the circulation of the placenta) or may represent the primary disorder, often with a hitherto unknown etiology. The worst consequence of a maturation disorder is the loss of the reserve capacity of a placenta with regard to oxygenation of the fetus with consecutive intrauterine phases of fetal hypoxia, in the worst case a late intrauterine fetal death.

Intermediate Villus Deficiency

A given gestational age is characterized by a typical mix of the three types of villi. When looking at the placental tissue under the microscope, the number of intermediate villi seems to be reduced in "intermediate villus deficiency". The marked jump in caliber between the inconspicuous stem villi and an impressively greatly increased number of terminal villi (◘ Fig. 3.14a) with small caliber and increased vascularization is striking. The color of the placental tissue visible to the naked eye, especially when viewing the sectional surface of the parenchyma, is due to the proportion of the cross sectional area of fetal blood vessels. Although circumstances of delivery (time of cord clamping) play a role, it is generally true that a placenta will appear progressively redder as pregnancy progresses. Deviating from this, a placenta e.g. of the 28th week of gestation from a pregnancy with preeclampsia will show a dark red hue—actually only typical for a term placenta -, caused by the high proportion of fetal vessel cross-sections in the total area of the placental parenchyma. The perfusion disturbance of the placenta originating from the maternal side in preeclampsia has led to infarctions and reduced growth of the placenta. Obviously, this is compensated from the fetal side by an increase in performance through maturation with premature differentiation and increased vascularization of the terminal villi. This leads to the morphological picture of a dark red placenta and "consumption" of the growth compartment of the intermediate villi.

Chorangiosis

Chorangiosis refers to an absolute increase of blood vessels in the area of the intermediate and terminal villi. In the quantitative analysis of the cross-sections of terminal villi, up to ten vessel cross-sections per terminal villus are counted in the most severely altered areas. In this maturation disorder the functional significance of this increase is unclear. Excessive fetal vascular growth does not seem to improve the gas exchange between maternal and fetal blood, presumably because the diffusion distances remain far. Chorangiosis type I with increase in capillaries and deficient formation of syncytio-capillary membranes is distinguished from chorangiosis type II with sinusoidally dilated vessels and numerous syncytio-capillary membranes (◘ Fig. 3.15b). At the very least, chorangiosis type II appears to be a feto-placental adaptive attempt, as placentas from mothers who go through pregnancy and delivery at high altitude have a much higher incidence of chorangiosis. Late intrauterine deaths with chorangiosis as the sole finding are very rare. The term chorangioma should be distinguished. It is a circumscribed (not diffuse) change of the placenta in the sense of a hemangioma in the chorionic villi.

Maturation Retardation

From the 34th week at the latest, the small caliber terminal villus with sinusoidal blood vessels and syncytio-capillary metabolic membranes must be by far the quantitatively dominant villous type (◘ Fig. 3.15a). If intermediate villi with larger caliber, higher stromal content, and only rudimentarily developed syncytio-capillary membranes are quantitatively image-dominant during this time period, the term "villous maturation retardation" is used (◘ Fig. 3.15c). Often the maturation-retarded placenta is large with a sectional surface that is too pale for the gestational age. This is explained by the insufficiently forced vascularization of

the villi. This disorder affecting all aspects of the morphological maturation of a placenta evenly, is typical for inadequately treated diabetes mellitus, but otherwise rare. The functional consequence is the gradual reduction of the reserve capacity of the organ for oxygenation of the fetus, whereas the nutritive function is apparently not affected. The potential danger lies in the unexpected intrauterine death of a macrosomic child, which may occur in the last six weeks of pregnancy or in the period of a prolonged pregnancy.

Terminal Villus Deficiency

Microscopic examination reveals a regular branching of the chorionic villi with a quantitative dominance of small calibers. Only a specific analysis of the villi reveals that the morphology of a mature terminal villus is rarely found. In an ideal-typical normal terminal villus, three sinusoidally transformed vessels can be recognized that occupy about 30% of the stroma and form long-stretched syncytio-capillary membranes (◘ Fig. 3.15a). In terminal villus deficiency, capillary blood vessels are found in which a tendency to sinusoidal dilation is evident but has resulted in a regular syncytio-capillary membrane on average only once per terminal villus (◘ Fig. 3.15d). Since the ideal-typical terminal villi occur far too rarely, the name "terminal villus deficiency" results. As far as the maturation of the placenta is determined by the picture of villous branching, this seems to be undisturbed. Typical is the contrast to the clearly retarded vascularization, from which the term "dissociated villous maturation disorder" is derived. The entire exchange of substances between fetus and mother takes place at the surface of the chorionic villi. From the 34th week of gestation, a differently differentiated surface of the terminal villi becomes important; the syncytio-capillary membranes conduce to gas exchange. An insufficient proportion of this compartment reduces the reserve capacity of the placenta in this respect. Therefore, terminal villus deficiency has the same functional hazard potential as diabetes-associated maturation retardation. In two out of 1000 pregnancies, terminal villus deficiency leads to late intrauterine fetal death; the etiology is unknown. If there were an easily identifiable parameter indicating this disorder (e.g., ultrasound morphology of the placenta or hypoxia-associated measure in maternal blood), earlier induced delivery could significantly reduce perinatal mortality.

Responses and Evidence from Studies of Small-for-date Newborns and Stillbirths

In most cases, a pathology of the placenta can be detected in newborns born alive but dystrophic. The need for examination results from the perceived need for explanation, possibly also from the need to support a decision made (e.g. caesarean section) by placental findings. Whether a placenta was too small for a given gestational age is easily answered using the norm chart. However, the diagnosis of placental insufficiency only arises when a pathological process within the placenta is evident. Otherwise, it could be a small placenta with a small child for other reasons (e.g. a chromosomal disorder such as uniparental disomy).

The retrospective decision as to whether the fetus could have been expected to endure a further intrauterine period or whether it was high time to free it from unfavorable (placental) circumstances is hardly possible on the basis of placental morphology. Should the assumption of fetal intrauterine hypoxia conditions have led to a premature delivery, the maturation disorders (chorangiosis, maturation retardation and terminal villus deficiency) in particular should be considered in addition to the possibility of an umbilical cord complication or circumscribed premature placental abruption. Of all fetuses whose placenta has maturation retardation, few die from hypoxia in utero

(2.3%). However, the risk of fatal hypoxia is increased 70-fold compared to morphologically normal placentas.

3.2.5 Postpartum Period

This section deals with problems that arise only in the placental period (e.g. incomplete detachment). It also deals with the placenta as the origin of neoplasia and finally it deals with statements that can be made about twins on the basis of the placenta.

Placenta Accreta or Increta

When commissioned to examine a placenta, the pathologist is rarely asked to check the completeness of the placenta; presumably because this is the classic task of the midwife and obstetrician. If manual dislodgment of the placenta has been necessary, the placenta is usually obviously incomplete or so lacerated that assessment of completeness is not possible. In this situation, the question arises whether the need for manual dislodgment can be explained by implantation pathology. The pathologist is faced with the dilemma that the most informative tissue—in the case of incomplete detachment—is probably still within the uterine cavity. An attempt is then made to examine in increased quantity those basal portions of the placenta which do not apparently correspond to an opened intervillous space. In normal implantation this region shows a zone of decidua several millimeters wide. In the basal sections of a not spontaneously detached placenta, however, smooth muscle fibers are frequently found in the immediate vicinity of placental villi (placenta accreta) or chorionic villi between uterine muscle cells (placenta increta). If this finding cannot be made, this by no means excludes a placenta accreta/increta, since—at least in the case of a clearly incomplete placenta—it can be argued that the relevant finding is still in utero.

Neoplasia

Malignant Degeneration of the Chorial Tissue

The chorionic epithelium can transform malignantly; choriocarcinoma develop in 1:25,000 to 1:40,000 pregnancies, with the relatively highest incidence observed in preceding complete hydatidiform moles. Choriocarcinoma arising from placental tissues grow very rapidly. However, it is easily treatable, probably because it represents a partial allograft for the maternal organism due to the paternal genetic material it contains and is therefore immunologically vulnerable. Rarely, choriocarcinoma are diagnosed in an otherwise inconspicuous placenta after term delivery. The indication for the examination of an affected placenta arises retrospectively from a focal finding which, after possible imaging as well as macroscopically, appears to be a solitary infarct. According to histological criteria, the highly atypical and highly proliferative tissue is systematically best described as an in situ choriocarcinoma. Towards the intervillous space it shows necrosis covered by fibrin, the fetal villous stroma is usually not infiltrated. Intrauterine metastasis to the fetus is very unlikely.

The manifestation of choriocarcinoma in the maternal organism can typically occur even after a long latency, especially in the form of lung and/or brain metastases. Chemotherapy is still very effective even then. Differentially, placental site trophoblastic tumors, which arise from the extravillous trophoblast that physiologically infiltrates the maternal uterine wall during pregnancy, should be distinguished. This neoplasia shows low chemosensitivity. Therapy has a chance of success if complete surgical removal is successful.

Metastases to the Placenta

With increasing maternal age, pregnancies are also more frequent (1:1000), in which a perhaps initially unknown malignant dis-

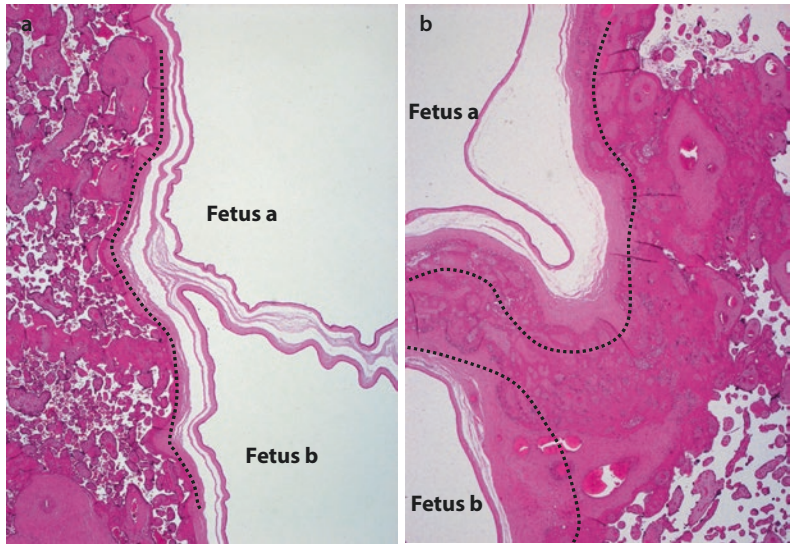
ease of the mother is present. Metastasis to the placenta is very rare; by far the most common is the presence of cells of a malignant melanoma in the intervillous space, but carcinomas of the cervix, breast, lung and gastrointestinal tract also occur. By the time of birth, malignant disease has practically always become manifest in the mother. Metastasis to the fetus is even rarer; metastases of melanoma, lymphoma/leukemia, and adenocarcinoma of the lung have been observed. An indication for the examination of the placenta in case of known malignant disease of the mother can be derived from this; the metastases are usually not visible macroscopically, but have to be detected by abundant sampling.

Rarely, the malignant disease may be primary in the fetus and thus cells of a neuroblastoma, a teratocarcinoma or a leukemia may be visible in the fetal circulation in the placenta.

Twins

Chorionicity and Zygosity

The partition between the amniotic cavities of twins may be transparent or opaque. In the case of a common chorionic cavity ("monochorial"), the partition is transparent because it consists only of two layers of amnion (without intervening chorionic tissue) (◘ Fig. 3.16a). In this situation, one can read a newspaper through the stretched partition. This test for transparency proves the monochorial state and thus the monozygotism of the twins. If the fetal membranes are opaque and the newspaper cannot be read through the stretched partition, this is due to the additional chorionic tissue between the amniotic covering layers (◘ Fig. 3.16b). The finding is "dichorial twin pregnancy". In this case, no statement on the zygosity is possible for the individual case.



◘ **Fig. 3.16** **a** "Monochorial means monozygotic": If the partition between fetus A and B does not contain any chorionic tissue (*dashed line*), the twins originate from one zygote ("identical"). The tissue of the separating wall is slidable on the chorionic plate of the placenta and is relatively clearly transparent when stretched. **b** "Dichorial is not informative" (concerning zygosity in individual cases): If the separating wall between fetus A and B contains chorionic tissue (*dashed line*), the twins may be identical or fraternal twins. The tissue of the partition is fixed on the chorionic plate of the placenta and is opaque even when stretched. (H&E, 50×)

Fetofetal Transfusion

The dividing wall between the amniotic cavities of identical twins does not necessarily originate where the two placentas meet. Rather, the border area is a kind of watershed between the branches of the blood vessels located in the chorionic plate, which cover the chorionic plate arising from the two umbilical cord insertions. Arteries and veins may be distinguished by the fact that at the crossing points the arteries pass over the veins. Vascular connections virtually always exist between the fused placentas of identical twins. Large caliber arterio-arterial and veno-venous connections within the chorionic plate can be identified as such to the naked eye. Functionally, they can be beneficial if simultaneously existing arterio-venous connections in the parenchyma of the placenta form a unidirectional shunt and the resulting volume shift can be balanced out via the large-caliber connections. However, if a rapid drop in blood pressure occurs in one of the fetuses (e.g. in the case of hypoxia or premature abruption), the large-caliber connections can lead to a volume shift that is fatal for both partners in the short term.

With a little practice, unidirectional shunts at the level of the villous parenchyma can be recognized by following the extensions of the vascular system in the chorionic plate. Shunts are found in areas which are supplied from one side by an arterial vessel and where a venous vessel of approximately the same size is not directed towards the same side but to the opposite side. Since in these shunts the blood can only flow from the arterial to the venous side, there will be several such areas with reversed directions of flow. Since the balance of the blood flows can hardly be the same, the large-caliber connections allow the (saving) volume balance when blood pressure differences arise. If the large connections are missing, the basis for a chronic fetofetal transfusion syndrome is laid.

3.3 Biobanking

Berthold Huppertz

3.3.1 Introduction

Since the beginning of the 1980s, there has been a steady increase in scientific publications on the subject of "biomarkers and the placenta". Meanwhile, there are almost 6000 publications on the topic. This means that a large number of data and hypotheses are available on which biomarker is the better one, for which pathology it is specific and at which time it is ideally used. However, at the same time it has not been questioned, or only rarely, whether the samples used for the identification, testing and validation of these markers meet appropriate quality standards.

It was not until the beginning of the 2000s that the value of systematic sample collections was recognized—and with it the necessity of a field that deals with "pre-analysis". Accordingly, the increase of these publications started only after the year 2000 and has currently by far not reached the numbers of placental biomarkers (maximum 143 compared to maximum 393 for "Biomarkers and Placenta"). If only the topic "biomarkers" is taken into account, almost 60,000 publications (exactly 59,991) can be found in PubMed in 2015 alone. All of these studies should also necessarily deal with pre-analysis.

What Exactly Is Pre-analysis?

The pre-analytical phase is the period between the sample collection (e.g. blood drawing) and the analysis of the sample. The handling of the sample between these two events significantly determines the quality of the analysis result. More and more attention is being paid to quality in analyses to achieve the best possible results. Also, the era of omics technologies has made it pos-

sible to analyze smaller and smaller amounts and more components of a sample. But do the samples used for this also meet the high demands of these technologies?

Example: Imagine a serum sample in which a specific protein hormone is to be determined. This sample was taken on a morning, then treated according to the specifications in the clinic and then left in the department to be picked up by the transport service. This service overlooked the sample and did not pick it up and took it to the lab only the next day. There, the hormone level was determined and appropriate therapy was initiated for the patient.

It is rather unlikely that the therapy will be adequate for the patient.

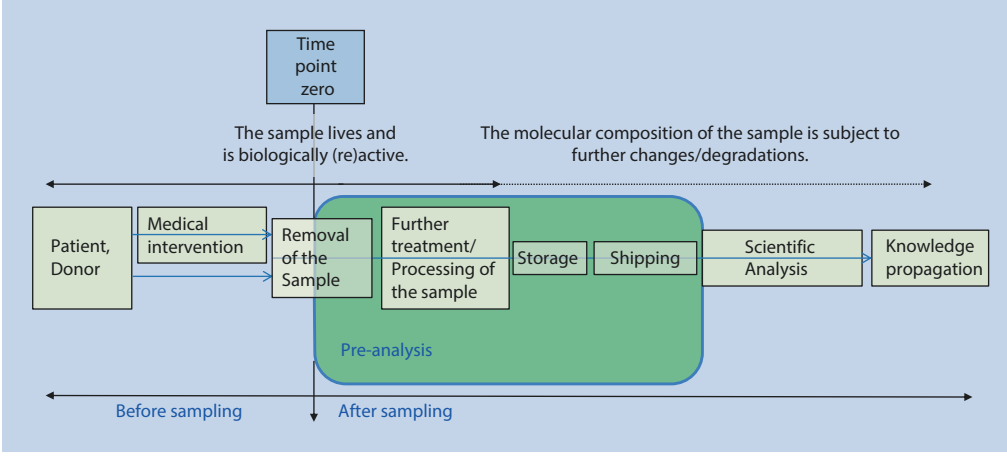
This example is taken from clinical laboratory medicine, but of course applies just as much to any scientific analysis. Here, very few laboratories have a quality control system in place to check whether the samples used meet appropriate quality criteria that are necessary for the study. This is one of the reasons why in the scientific literature the data on many biomarkers are so very different and contradictory.

3.3.2 Variables Affecting the Composition of a Sample

Although the pre-analytical phase begins with the collection of the sample, other factors that influence the composition of the sample must be taken into account. Looking at the time course in ◘ Fig. 3.17, it can be seen that even before a sample is taken (tissue, fluid, cells, etc.) a wide variety of factors can have a significant influence on the sample. Therefore, these variables are divided into two groups, the variables before and after sample collection.

Variables Before Sampling

The variables that can have a decisive influence on the sample before collection are, on the one hand, to be sought in the donor's lifestyle and concern diet, consumption of drugs (nicotine, alcohol, etc.), intake of medication, etc.. Other variables include environmental factors such as air pollution, contact with chemicals, etc. In addition, there are variables that are directly related to the collection of samples. This is particularly true in the case of surgeries.



◘ **Fig. 3.17** The pre-analytical phase. Schematic representation of the pre-analytical phase between sample collection and analysis. After a sample has been taken, a large number of variables can influence the quality and degree of preservation of a sample

Example: A tumor is to be removed during a surgery. For this purpose, the blood supply to the corresponding organ must be significantly restricted or stopped completely before the tissue is removed. From now on, this organ is undersupplied and runs into ischemia. Since this undersupply of the organ still occurs in the body and thus at body temperature, this is referred to as "warm ischemia". In addition, the patient has been administered medication, is under anesthesia and the blood pressure could also vary significantly.

All of these variables prior to retrieval (warm ischemia time, medication, anesthesia type and duration, etc.) affect the tumor tissue that is removed during surgery. The RNA and protein profiles of the tissue may undergo significant changes during this time due to oxygen deprivation alone, such that even immediately after removal, the tissue no longer fully resembles the tissue that was present in vivo prior to surgery. Since these variables are purely present to help the patient prior to retrieval, no scientific study should provide guidelines here. However, the relevant variables can be documented, especially the warm ischemia time. Thus, comparison of these times may explain divergent analysis data.

Variables After Sampling

The pre-analytical variables after sample collection are variables that have a decisive influence on the results of all further examinations and analyses and can be considered independent of the patient. In addition, the researchers can have a direct influence on these variables. The time between sample collection and analysis is referred to as the "cold ischemia time", as this is when the sample has left the body and is usually processed, transported and stored at temperatures lower than body temperature.

This time, it is about sample handling, which includes the following:

- Direct processing of the sample after collection,
- Transport of the sample from the collection site to storage (or directly to analysis),
- Storage of the sample (short, medium, long term),
- Transport from storage to analysis,
- Handling of the sample before analysis.

During the time window between collection and analysis, a sample can be exposed to a variety of influences. This can lead to massive changes in the sample and thus significantly influence the analysis results. Since these variables are within the direct sphere of influence of the researcher, it is necessary to develop criteria before the start of sample collection that enable the best possible preservation of sample quality during the pre-analytical phase. If it is already known before sample collection which analyses are to be carried out, the criteria for sample handling can be specifically defined.

3.3.3 Collection or Biobank?

Researchers in the medical field have been collecting human specimens of interest for centuries. The importance of human tissue collections was politically recognized in Austria more than 200 years ago. In 1811, the Austrian Study Court Commission (Studienhofkommission) issued a decree for the "establishment and maintenance of anatomical-pathological cabinets". These cabinets, i.e. anatomical-pathological collections, were to be established to promote the teaching of medical students at all medical-surgical teaching institutions. In addition, physicians were expressly obliged "… in all cases in which, in their clinics or in the hospitals and maternity homes entrusted to them, the opportunity arises to obtain curious anatomical-pathological specimens, games of nature, etc., to collect these or through their assistants and to hand them over to the cabinets". The specimens in these collections were mainly fixed and preserved

Table 3.1 Comparison between individual collections and biobanks

	Individual Collection	Biobank
Collection	By an individual or a research group	Through several research groups or centrally at a clinic/university
Sample collection	For a particular study	For many possible studies not yet defined at the time of collection
Ethical vote and donor consent form	Specific to a study (if available)	Broad for use of samples/data for e.g. biomedical research
Sample diversity	Low	High
Labeling of samples	Handwritten	Barcodes
Creation of databases	Handwritten	Automated
Quality monitoring of samples and sample storage	Rarely present	Generally available
Collection of samples and documentation of data	In the hands of non-specifically trained persons	In the hands of appropriately trained persons
Systematic guidelines (SOPs) for sample and data handling	Rarely present	Generally available

SOP Standard Operating Procedure

with alcohol or formalin. Since—as described above—mainly "games of nature" were collected here, mainly curiosity collections developed, which can still be admired today in many anatomical collections.

Towards the end of the last century, the targeted collection of further samples began, not only to fix them in formalin but also to store them frozen. Thus, the spectrum of collections expanded to include liquid samples, especially blood samples, and tissue samples stored at ultra-low temperatures (liquid nitrogen). However, it is still the individual researcher who starts these collections and supervises the storage of the samples. These collections are still operated in this way today.

It was not until the 21st century that collections were taken to the next level and the term "biobank" was coined. Biobanks are fundamentally different from individual collections of researchers, as they involve a highly organized, systematic collection of samples with their associated data that are made available for research purposes. Although biobanks have a very wide range of foci, there are fundamental differences between individual collections and biobanks. Individual collections by researchers or small groups of researchers have a number of disadvantages compared with biobanks, in which samples are collected, stored and made available for research systematically and on the basis of standardized processes (Table 3.1).

Reasons for Creating a Biobank

The reasons for the emergence of biobanks arise from the following problems with individual collections.

- Many samples that are available in individual collections cannot be used for other studies after the respective studies have been completed, because

no further informed consent has been obtained from the donors.
- The labelling of the samples is often no longer comprehensible, so that samples can no longer be assigned.
- Due to the manual labeling of the samples and also manual input of data, the assignment of samples to data is extremely error-prone.
- Data security is rarely given, since the data assigned to the samples are often stored on storage media that are not backed up multiple times.
- The variability of sample quality is very high:
  - due to the lack of specific protocols,
  - due to the lack of monitoring of sample storage,
  - due to improper handling of samples (e.g. multiple freeze-thawing of blood samples).

For these and other reasons, biobanks were established in many countries to address and prevent fundamental problems in sample and data collection and storage. The first publication in PubMed on the topic of biobanking was published 1996 and by the year 2000 there were a total of 5 publications with the keyword biobank.

Definition of Biobanks

To date, the term biobank is neither protected nor clearly defined. Many institutions have attempted to define it, so that today a large number of definitions can be found, some of which overlap but differ in detail. Two examples are intended to demonstrate this diversity:

- Sweden, 2003: The Swedish Ministry of Health defined the term biobank in 2003 as "biological material from one or more human beings that is collected and preserved for an indefinite or limited period, and whose origin is traceable to an individual or individuals".
- USA, 2016: The NIH published its definition of a biobank in July 2016: "A biobank is a repository that stores and manages biological samples known as biospecimens for use in research."

These two definitions illustrate the differences and overlaps very well. There is general agreement that biological material is collected and stored in biobanks—even if the species of origin of the samples is viewed differently. Biobanks generally contain biological samples. This means—in contrast to the definition in Sweden, where only human samples can be found in a biobank—that these samples can originate from plants, animals (including humans), fungi but also microorganisms. In the definition from Sweden it is not clear why these samples are collected and stored, research is missing in this definition. In addition, both definitions lack a statement about the data that can be assigned to the samples. Without these data, a sample has little or no value for research.

To obtain a comprehensive definition of biobanks, the following definition can be used.

> Biobanks are facilities for the systematic collection, storage and distribution of high quality biological samples and their associated (clinical) data for research, embedded in an ethical and legal framework.

When considering human biobanks for medical research (such as clinical biobanks), these must be distinguished from collections of samples and data for other purposes. Such collections include collections for therapeutic or forensic purposes, pure medical archives for documentation, and the historical anatomical/pathological collections and museums.

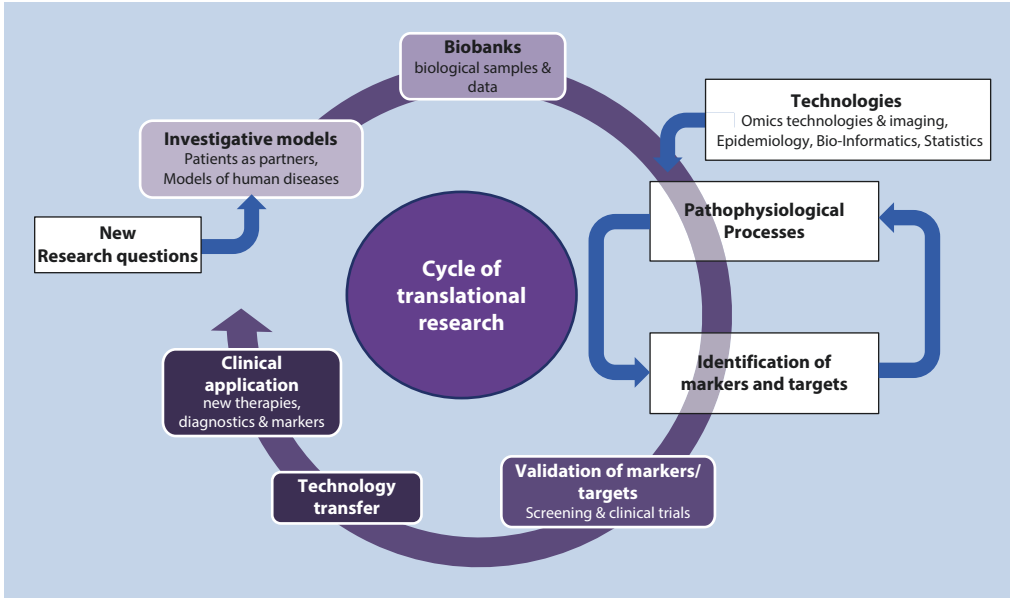
Advantages of Biobanks

It was only through the development of biobanks and the implementation of corresponding quality standards that the field of new omics technologies was able to develop so rapidly. In the case of individual collections, it became apparent that the differences in quality of the samples within a collection, and especially between two or more collections, are so serious that it is no longer possible to make a clear statement about the analysis data. Analyses with samples from several collections showed that, based on the analysis data, it is easier to distinguish the collections from each other than the control samples from the disease samples.

The ongoing development of quality standards in the field of biobanks is coupled with an increasing number of studies that focus on the effects of the pre-analytical phase on sample quality. One EU-funded project that has been instrumental in the development of quality standards is the SPIDIA project ► (► www.spidia.eu). As part of this project, not only were samples from individual biobanks examined for effects during the pre-analytical phase, but interlaboratory tests were also carried out with several biobanks to identify comparabilities between biobanks.

Today, the difference between individual collections and biobanks with regard to the quality of samples and data as well as ethical and legal issues has become so clear that some countries, such as Switzerland, have passed corresponding biobank laws that only permit the collection of samples and their distribution for research in and from registered biobanks. As a result of this development, biobanks are becoming increasingly important in the field of precision medicine.

This significance of biobanks in the cycle of translational research is illustrated in ◘ Fig. 3.18. It was only through the development of biobanks that the potential of omics technologies could be exploited. The data depth of these technologies revealed how crucial the pre-analytical phase is for



◘ **Fig. 3.18** The cycle of translational research. Only the combination of high sample quality in biobanks and the development of new technologies such as omics technologies has led to a significant improvement in the identification of markers and targets. This accelerates technology transfer and helps to find diagnoses and new therapies for patients more quickly

the analysis and thus for the results. Only through the combination of biobanks and omics technologies is it now possible to identify a large number of new markers and potential starting points for therapies. Subsequent validation and the corresponding technology transfer then enable precision medicine to be translated into benefits for patients (Fig. 3.18).

Further Readings

Further Reading on Section 3.2

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Placental Function—Nutrient Transport—Gas Exchange

Michael Gruber, Birgit Hirschmugl, Carolin Schliefsteiner and Christian Wadsack

Contents

B. Huppertz, E. Schleußner (eds.), *The Placenta*, https://doi.org/10.1007/978-3-662-66256-4_4

4.1 General Functions of the Placenta

Just as the placenta develops and grows during pregnancy, its functions also change during gestation.

In the first three months of pregnancy, the interaction between the early placenta and the maternal decidua, which arises from the endometrium, is particularly crucial for the implantation of the embryo and for maternal tolerance to the embryo and fetus. Both the decidua and placenta contain immune cells that provide immune system adaptation to pregnancy, such as natural killer (NK) cells, macrophages, and regulatory T cells. With regard to the immunological function of the placenta, reference is made to ▶ Chap. 2.

The placenta is a multicellular organ and, in addition to its immunological function, also fulfils an endocrine function in that it responds to hormonal signals from the mother and child and also stimulates the production of certain pregnancy hormones which are essential for the maintenance of the pregnancy, the development of the fetus and the (timely) induction of birth. These functional aspects are discussed in ▶ Chap. 5.

However, the most essential functions of the placenta are the supply of nutrients to the fetus and the exchange of gases between maternal and fetal blood. These functions will be discussed in detail in the following sections. Crucial to the transport of nutrients and oxygen is the hemochorial villous architecture of the placenta, which maximizes the surface area available for exchange (▶ Chap. 1), and a switch in maternal metabolism. During the first half of pregnancy, the maternal metabolism corresponds to an anabolic situation, i.e., in addition to the energy expenditure for the growth of the placenta and fetus, energy from food is primarily used to build up energy reserves (glycogen stores in the liver, fat depots). From the 20th week of pregnancy onwards, logarithmic growth of the fetus sets in and the majority of nutrients need to be made available to the child—on the one hand through increased food intake, and on the other hand through a change in maternal metabolism to a catabolic situation. Although up to three times more insulin is produced by the mother compared to the non-pregnant state (Freinkel 1980), sensitivity towards insulin decreases by about 50% (Catalano et al. 1991; Buchanan et al. 1990). Thereby, postprandial glucose and amino acids are supplied directly to the fetus and, in addition, energy is mobilized from maternal fat stores in the fasting state. The functions of the placenta during pregnancy are summarized in ◘ Fig. 4.1.

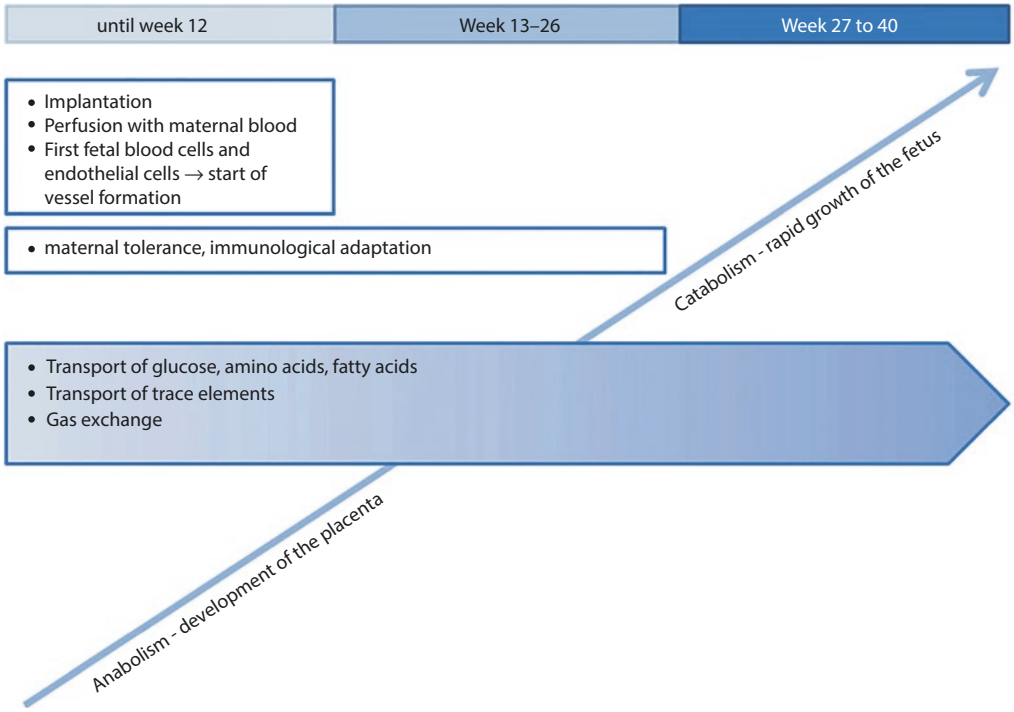

Fig. 4.1 Functions of the placenta during pregnancy

4.2 Nutrient Transport Across the Placenta

4.2.1 Transport of Lipids and Fatty Acids

One of the main tasks of the placenta is the transport of nutrients from the maternal blood to the fetus to ensure its development and growth over the duration of pregnancy. In particular, the transport of lipids across the placenta plays an important role.

In the first half of pregnancy, maternal hyperphagia leads to the build-up of maternal fat reserves in the white adipose tissue. In the course of a healthy pregnancy, the maternal lipid profile changes. Especially in the last trimester of pregnancy, increased fatty acids are mobilized from maternal lipid stores, leading to pregnancy-induced hyperlipidemia. This excess supply of maternal lipids is available to the fetus for growth and for the formation of its own fat reserves after transport across the placenta (Herrera et al. 2006). Specifically, fatty acids are the major basic building blocks for the establishment of fetal lipid reserves. The transfer of fatty acids across the placenta largely follows a maternofetal concentration gradient, and only non-esterified, so-called free fatty acids are transported. However, free fatty acids account for only about 1% of the total fatty acids present in maternal plasma. The majority of fatty acids present in plasma are esterified in the form of triglycerides, phospholipids and cholesterol esters, and 99% of these are bound in lipoproteins.

These fatty acids bound in lipoproteins must first be prepared for uptake into the placenta, since phospholipids and triglycerides cannot cross the placenta directly. At

least two processes are at issue for the provision of fatty acids at the placental barrier. First, the uptake of triglyceride-rich lipoprotein particles via lipoprotein receptors on the syncytiotrophoblast and the further hydrolysis of fatty acids by intracellular lipases (Desoye et al. 2011). Second, the release of fatty acids at the microvillous membrane of the syncytiotrophoblast by extracellular lipases, such as endothelial lipase (Gauster et al. 2007) or lipoprotein lipase (Waterman et al. 2000), and the uptake of the free fatty acids into the syncytium. Both diffusion and protein-mediated processes are thought to be responsible for the uptake of free fatty acids into the syncytiotrophoblast layer. In particular, the so-called fatty acid transport proteins (FATP 1-6, "fatty acid transport proteins"), fatty acid translocase (CD36, also FAT, "fatty acid translocase") and intracellular fatty acid binding proteins (FABP 1-12, "fatty acid binding proteins") are involved in the cellular uptake of long-chain free fatty acids (≥16 carbon atoms in the fatty acid chain). Whether there is a preferential interaction between these transport proteins and fatty acids with different chain length or degree of saturation is still under debate.

In addition, the exact sequence of the uptake processes of fatty acids across cellular membranes has not yet been fully elucidated. However, once fatty acids from maternal plasma have entered the syncytiotrophoblast, an intermediate esterification of these fatty acids to triglycerides and phospholipids may occur. On the one hand, the phospholipids and triglycerides generated in this way represent a placental metabolic reserve, for example, for energy production by β-oxidation or for the provision of phospholipids for the expansion of cellular membranes. On the other hand, fatty acids can be hydrolyzed by lipases from placental triglycerides and phospholipids and thus be made available again for transport to the fetus (Herrera and Desoye 2016). Maternofetal transport of fatty acids favors essential fatty acids of the omega-3 and omega-6 family and their long-chain polyunsaturated fatty acid derivatives, such as docosahexaenoic acid (Haggarty 2010). This preferential transport results in an accumulation of docosahexaenoic acid in the fetus compared to maternal plasma. This observation highlights the important role of omega-3 and omega-6 fatty acids, such as docosahexaenoic acid, in fetal optic nerve and brain development (Innis 2005). However, the mechanisms underlying maternofetal fatty acid transport, including the protein factors involved, and the regulation of fatty acid transport in the placenta remain not fully elucidated and are currently the subject of numerous research projects.

4.2.2 Transport of Glucose

Glucose is an essential energy source for the basic needs of the fetus because fetal glucose production is minimal (Kalhan et al. 1979). Accordingly, the glucose concentration in maternal blood is usually higher than in fetal blood, so that the glucose flow follows a gradient from mother to child (Aynsley-Green et al. 1985; Bozzetti et al. 1988), with a linear relationship between the glucose concentration in maternal and fetal blood (Whaley et al. 1966; Tobin et al. 1969).

The transport of glucose across the placenta is ensured by facilitated diffusion, so that no active transport that consumes additional energy is required. Nevertheless, facilitated diffusion requires glucose transporters (GLUT) that transport glucose across the microvillous and basal membranes of the syncytiotrophoblast in a Na^{2+} -independent manner. Three such transporters are known to exist in the placenta: GLUT1, GLUT3, and GLUT4. GLUT1 is the transporter found on all different cell types of the placenta (Hahn et al. 1995). GLUT3 is

expressed by fetal endothelial cells and placental stromal cells (Hahn et al. 2001). GLUT4 is found exclusively in placental stromal cells (Xing et al. 1998).

Essentially, glucose transport across the placenta is unregulated and therefore unlimited. The GLUT transport system has a high capacity and is virtually unable to saturate, which would require a glucose concentration in maternal blood of >20 mmol/l (Kalhan and Parimi 2000). Although GLUT1 is normally regulated by ambient glucose concentration, GLUT1 expression in trophoblasts in vitro is reduced only at concentrations >20–25 mmol/l (Hahn et al. 2000). Accordingly, transport is hardly regulated by transporter availability, i.e. not by diffusion. If anything, glucose transfer to the fetus is flow-limited, which has also been shown in studies with varying flow rates of maternal and fetal blood (Gilbert et al. 1984). It is therefore not surprising that in pregnancies with gestational diabetes, but also with fetal growth restriction, glucose transport is unchanged (Osmond et al. 2001; Challis et al. 2000), although the level of transport molecules changes.

Ex vivo perfusion of the placenta (► Chap. 15) can be used to study the transport of various nutrients, including glucose. For placentas from diabetic pregnancies, this method has shown that glucose transport is unchanged despite the disease (Osmond et al. 2001). Furthermore, there is also no glucose gradient between vein and arteries in the umbilical cord (Challis et al. 2000), also indicating unaltered transport. Rather, the fetal pancreas compensates for the high glucose level by overproducing insulin. However, since placental glucose transport is insulin-independent, this does not directly result in the transport of less glucose, but only in metabolizing the excess glucose, which results in a long-term change in fetal body composition, namely the buildup of additional fat mass (Durnwald et al. 2004). Glucose transport has also been studied in fetal growth restriction (FGR, "fetal growth restriction") using ex vivo perfusion (Challis et al. 2000). Although the placental turnover of glucose is increased in this case, the net transport is unchanged, since not only the weight of the fetus, but also the size and weight of the placenta are reduced in FGR, so that the transport remains proportionally the same (Jansson et al. 1993).

Not all of the glucose transported across the placenta is consumed by the fetus per se—the placenta itself is also a metabolically very active organ and about half of the transported glucose is metabolized in the placenta and stored as glycogen or lactate (Burd et al. 1975). The other half is thus directly available to the fetus. Although no direct measurements are possible in humans, it can be deduced from animal studies (e.g. in sheep) that the fetal rate for glucose consumption is about 5 mg glucose/kg body weight per minute (Kalhan et al. 1979). For oxidative metabolism, the glucose/O_2 quotient is an essential measure to estimate whether glucose alone is sufficient to meet energy requirements. The quotient is calculated by dividing the difference in glucose levels in venous and arterial cord blood by the difference in oxygen levels in these vessels. In humans it is 0.8 (Morriss et al. 1974)—thus glucose alone is not sufficient to meet the energy requirements of the fetus, and other sources of energy such as lactate and amino acids must be used in addition.

4.2.3 Transport of Proteins and Amino Acids

In addition to fatty acids and glucose, amino acids are an important source of energy for the growing fetus. Amino acids are not only important for building muscle mass, but are also building blocks for various biosynthetic pathways, e.g. the assembly of nucleotides and heme molecules. Impaired amino acid transport across the placenta has been asso-

ciated in studies with reduced fetal growth (Glazier et al. 1997; Jansson et al. 1998), defective neurological development (Leitner et al. 2007), and a higher risk of chronic hypertensive disease later in life (Barker 1998).

The transport of amino acids across the placenta is comparatively poorly understood, but data are available from molecular biological studies (expression of transporter molecules, etc.), as well as direct data on transport rates from studies using ex vivo perfusion of the human placenta.

In contrast to glucose, the transport of amino acids must occur via "real" transporter molecules with energy expenditure. Three steps are required for this: (1) uptake of amino acids into the trophoblast via transporters at the microvillous membrane; (2) transport/turnover in the cytosol; and (3) release of amino acids at the basal membrane of the trophoblast and diffusion into the fetal circulation. Several amino acid transporters are expressed in the placenta at least at the mRNA level. Whether these mRNAs are actually transcribed to protein is unclear in many cases. Moreover, not only the presence per se, but also the subcellular localization, e.g. on the microvillous or the basal membrane of the syncytiotrophoblast, is essential.

There are two main types of amino acid transporters, both of which are found in the human placenta: (1) accumulative transporters, which transport amino acids into the cell and increase the amino acid concentration in the cell, and (2) amino acid exchangers, which transport a particular amino acid only in exchange for another amino acid, thus keeping the amino acid concentration in the cell the same but changing the amino acid composition in the cell, this is essential e.g. for the uptake of essential amino acids into the cell.

At the microvillous membrane of the syncytiotrophoblast, there are predominantly accumulative transporters that actively transport amino acids against a concentration gradient into the trophoblast. These amino acids can then be further transported by amino acid exchangers in return for other amino acids. Accumulative transporters at the microvillous membrane include members of several transporter families, e.g. the system A family, which transports small neutral amino acids, or the system Y-AG, which mediates the uptake of glutamate and aspartic acid.

Although the principle of uptake at the microvillous membrane is well documented, much less is known about transport across the basal membrane to the fetus. Both accumulative transporters and exchangers are present on the basal membrane. However, accumulative transporters can only take up amino acids and are therefore unlikely to be involved in the release of amino acids into the fetal circulation. Exchangers are able to mediate both uptake and release of amino acids, but can only change the composition of the amino acid pool in the cell, not the absolute concentration of amino acids. Continuous transport of amino acids to the fetus is necessary for its growth, so transporters other than amino acid exchangers that can release amino acids must theoretically be present at the basal membrane.

Ex vivo placental perfusion can also be used to study the transport of amino acids. Radioactively labelled amino acids are used for this purpose. Since amino acid exchangers can only work if amino acids are present in the fetal circulation, transport across the basal membrane can be studied independently of exchange mechanisms by not offering amino acids on the fetal side during perfusion. After a certain time, amino acids can then be added to the fetal reservoir to additionally study transport via exchangers (Cleal et al. 2007). Such studies have demonstrated that predominantly exchangers of the alanine-serine-cysteine (ASC) and L systems are localized to the basal membrane (Kudo and Boyd 2001;

Cleal et al. 2007; Okamoto et al. 2002). It has also been demonstrated that the two neutral amino acids L-serine and L-glycine, as well as L-leucine, are transported across the basal membrane independent of amino acid exchangers. Possible proteins that move amino acids across the basal membrane independent of amino acid exchange mechanisms could belong to system-N. The recently discovered amino acid transporters TAT1, LAT3, and LAT4 could also perform this role. TAT1 and LAT4 are expressed at the mRNA level in the placenta; however, data on protein expression and exact localization are not yet available.

Impaired amino acid transport has been associated with fetal growth restriction (FGR) in several studies (Mahendran et al. 1993; Jansson et al. 2002b). This particularly concerns the transport of essential amino acids (Bajoria et al. 2002; Cetin et al. 2005) such as leucine and phenylalanine (Paolini et al. 2001). What regulatory mechanisms underlie amino acid transport that may be relevant in FGR is poorly understood. Endocrine signals from both the mother and the fetus could have a regulatory effect on transport.

Furthermore, the local activity of the renin-angiotensin system appears to play a role in the regulation of blood flow to the placenta. Interestingly, changes in the concentration of angiotensin II and oxygen modulate the activity of system A-amino acid transporters in the placenta (Shibata et al. 2006; Nelson et al. 2003). Glucocorticoids also had similar effects on system A activity in in vitro studies (Ericsson et al. 2005).

Alternatively, there is also the possibility that the placenta has a sensory function and regulates transport capacity and rates accordingly. For example, in cell culture models of isolated trophoblasts and placental explants, it has been shown that insulin, insulin-like growth factor 1 (IGF-1) and leptin have an effect on the uptake of amino acids into cells. In addition to growth factors, the placenta could also act as a sensor for nutrient availability and regulate transport accordingly. Data from mouse models have shown that when amino acid availability in the mother is low, transport to the embryo is also down-regulated (Jansson and Powell 2006). Conversely, when particularly abundant nutrients are available, e.g. maternal hyperglycemia, amino acid transporters in the placenta are up-regulated (Jansson et al. 2002a). At the molecular level, the signal transduction pathway of rapamycin (mTOR) appears to be essential for nutrient sensing in many mammals; it controls cell growth depending on the availability of nutrients, e.g. branched amino acids. In the placenta, mTOR regulates the transport of L-leucine, and it has been shown that the mTOR system is down-regulated in FGR pregnancies (Roos et al. 2007), so that transport is reduced.

Figure 4.2 summarizes the most important transport pathways for macronutrients across the placenta.

4.2.4 Transport of Minerals and Trace Elements

The transport capacity for minerals and trace elements increases with the progression of pregnancy. In the case of calcium, magnesium and phosphorus, the plasma levels reached in the newborn exceed the plasma levels of the mother (Sibley and Boyd 1988; Schauberger and Pitkin 1979).

A continuous increase in **iron** transfer across the placenta is essential for fetal development and probably reaches a transfer rate of up to 7 mg per day in late pregnancy (Finch et al. 1983). Placental iron uptake occurs via Fe bound to transferrin $(TF)^{3+}$. A strongly increased expression of transferrin receptor 1 (TFR1) can be detected in the syncytiotrophoblast, which is only reached by cells in hematopoiesis

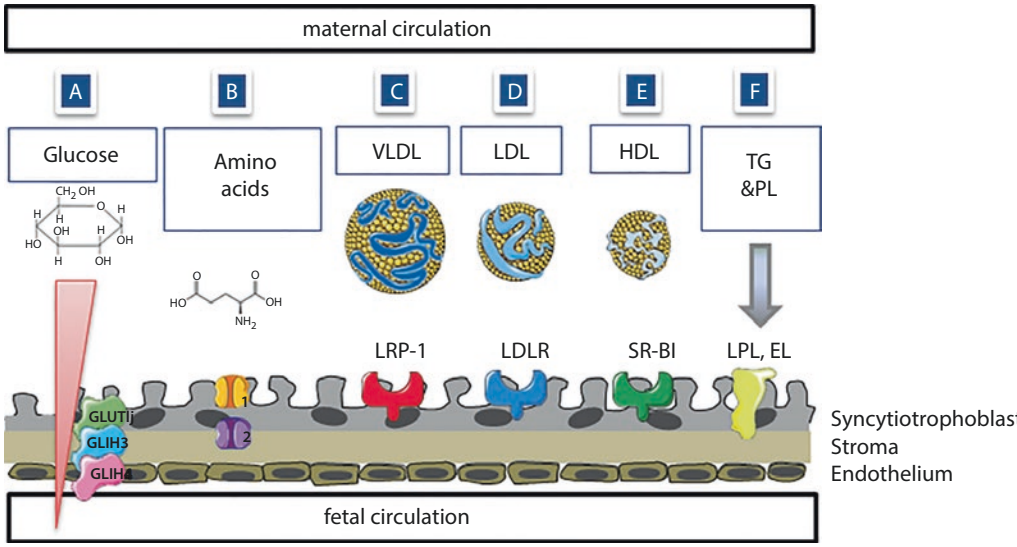

Fig. 4.2 Transport pathways for macronutrients across the placenta. **a** Glucose transport across the placenta follows a gradient from mother to child by "facilitated diffusion". The glucose transporters GLUT1 (*green*), GLUT3 (*turquoise*) and GLUT4 (*pink*) are each found ubiquitously, in the stroma or in the endothelium. **b** The transport of amino acids across the placenta occurs in two steps, which depend on different transporters on the apical side (1) and the basal side (2) of the syncytiotrophoblast membrane. Accumulative amino acid transporters and exchange transporters are distinguished. **c**, **d** Triglyceride-rich lipoproteins such as VLDL and LDL bind to their respective receptors in the placenta and are taken up by receptor-mediated endocytosis. The contained triglycerides are hydrolyzed by intracellular lipases and are available to the fetal circulation for synthesis processes or as energy storage. **e**, **f** Phospholipid- and cholesterol-rich HDL binds to the receptor SR-BI, which mediates the transport of maternal cholesterol across the placenta. Lipoprotein lipase and endothelial lipase are expressed in the syncytium and may also contribute to the release of free fatty acids and phospholipids from lipoproteins. *VLDL* Very low density lipoprotein, *LDL* Low density lipoprotein, *HDL* High density lipoprotein, *TG* Triglycerides, *PL* Phospholipids, *LPL* Lipoprotein lipase, *EL* Endothelial lipase, *LRP-1* Low density lipoprotein receptor-related protein 1, *LDLR* Low density lipoprotein receptor, *SR-BI* Scavenger receptor class B type I. (Adapted from Desoye et al. 2011)

(Ponka and Lok 1999). The Fe^{3+} -TF complex is taken up into the syncytium after binding with TFR1 in endosomes. The exact mechanism of the reduction of Fe^{3+} to Fe^{2+} in the syncytium is unknown. However, it is suspected that STEAP family proteins, which are detectable in the placenta, catalyze this reduction in the placenta, as such reduction has been shown in other cell types (Ohgami et al. 2006). The Fe^{2+} is excreted from the endosome by the transport protein "divalent metal transporter 1" (DTM1) or a protein of the zinc transporter family (ZIP) and transported intracellularly in the syncytium (Fleming et al. 1998; Pinilla-Tenas et al. 2011). Transport from the syncytium into the placental stroma and further transport into the fetal circulation is thought to be accomplished by ferroportin (Drakesmith et al. 2015) and ferritin (Maymon et al. 2000).

Calcium is required for the mineralization of the fetal skeleton and reaches a transport capacity of 140 mg/kg body weight per day at the end of the third trimester (Salle et al. 1987). This transport rate results in an amount of calcium of up to 30 g being transported across the placenta by the end of pregnancy (Lafond et al. 2001). This is achieved by sodium-calcium

pumps (Belkacemi et al. 2005) and calcium ATPases (Moreau et al. 2003) in the apical syncytiotrophoblast membrane. A calcium concentration gradient between maternal blood (2.23 mM) and the syncytium (0.1–1 μM) also has a favorable effect on calcium flux (Husain and Mughal 1992). This gradient is thought to be maintained by calcium-binding proteins such as calbindin-D-28 k (Belkacemi et al. 2003) and chaperone proteins such as clanexin (Tjoelker et al. 1994). A simultaneous buffering or storage function of these proteins is assumed to ensure a continuous supply of calcium to the fetus.

There is little evidence on how **magnesium** is supplied to the fetus via the placenta. It can be assumed that this is a calcium-independent energy-consuming process (Lourdes et al. 1992). In a mouse model, the magnesium transport protein TRPM6 has been linked to pregnancy outcome (Chubanov et al. 2016). In humans, a link between defects in the TRPM6 protein and hypomagnesemia has been shown (Voets et al. 2004). Overexpression of the SLC41A1 gene in preeclamptic placentas has been described and may suggest a key function of this magnesium transport protein in magnesium homeostasis (Kolisek et al. 2013). Further research in the field of placental magnesium balance will be necessary to adequately describe it.

Transport of **phosphorus** across the placenta is strongly sodium-associated, as in vitro studies have shown. Likewise, a pH and oxygen dependence has been demonstrated in the experiments (Lajeunesse and Brunette 1988; Lafond et al. 1988). The mRNA of two sodium-dependent phosphorus transporters, Slc20a1 and Slc20a2, has been detected in the placenta (Nishimura and Naito 2008). A detection at the protein level as well as an expression difference in preeclampsia substantiates the assumption that these two transporters are relevant in pregnancy (Yang et al. 2014).

The syncytiotrophoblast is thought to be the main regulator of **zinc** uptake (Mas and Sarkar 1991). A potassium-zinc transporter has been detected in the syncytial membrane (Aslam and McArdle 1992). Expression of mRNA for the following SLC30 family transporters has been demonstrated for the human placenta: ZnT1, 2, 4, and 5 (Ford 2004). As with iron transport, involvement of ZIP proteins in zinc transport is inferred from strong expression (Wang et al. 2012). Likewise, uptake of zinc bound to alpha-2-microglobulin into the placenta has been demonstrated (Douglas et al. 1998). The presence of the metal binding protein metallothionein in fetal amniotic and villous interstitial cells as well as the syncytiotrophoblast suggests that this protein is involved in the transplacental transport of zinc (Goyer et al. 1992). The regulation and fetal release of zinc are the subject of current research and are not fully understood.

4.3 Maternofetal Gas Exchange

The respiratory gases oxygen and carbon dioxide can be considered the most important gases to be transported across the placenta. Gas exchange is significantly influenced by hemoglobin.

The hemoglobin molecule consists of a heme group attached to the protein globin, the final molecule is a tetrameter and consists of four subunits. The main amino acid sequences of globin in human development are the five different chains, α-, β-, γ-, ε- and ζ-. The combination of these chains yields the differences between embryonic hemoglobin Gower-1 $\zeta_2\ \varepsilon_2$, Gower-2 $\alpha_2\ \varepsilon_2$, Portland-1 $\zeta_2\ \gamma_2$, Portland-2 $\zeta\ \beta_{22}$, fetal hemoglobin (HbF) $\alpha\ \gamma_{22}$, and adult hemoglobin (HbA) $\alpha_2\ \beta_2$.

All hemoglobins can adopt two conformations. The deoxygenated or t-form ("tense") and the oxygenated or r-form ("relaxed"). Oxygenation of one heme

group facilitates oxygenation of the remaining heme groups and results in a conformational change from t- to r-form. Crystallography shows that hemoglobin is more compact in the r-form (Perutz 1964). The two conformations can explain interactions with 2,3-bisphosphoglycerate (Perutz 1970) and the Bohr effect, which affect the oxygen binding affinity.

The hemoglobin-oxygen interaction depends on the oxygen saturation of the hemoglobin and the oxygen partial pressure. If these two terms are plotted on a graph, the sigmoidal equilibrium curve of oxygen is obtained, which is summarized by the term P_{50}. P_{50} describes a 50% saturation of hemoglobin with oxygen at pH 7.4 and body temperature. This P_{50} saturation is already reached at an oxygen partial pressure of 27 mmHg with HbA (Delivoria-Papadopoulos and McGowan 2011).

The sigmoidal shape of the oxygen saturation curve of hemoglobin can only be obtained if both of the following assumptions are made:

- the heme groups interact with oxygen in a defined sequence, and
- the heme groups influence each other in oxygen binding.

A regularity for this oxygen saturation property of hemoglobin was first described by Hill in the following equation:

$$y/100 = \frac{Kx^n}{1+Kx^n}$$

In the equation, y refers to the oxygen saturation in %, K describes the equilibrium constant, x corresponds to the oxygen partial pressure and n to the average number of iron atoms involved in oxygen binding (Hill 1910). Normal HbA has an n value of approximately 2.9. HbA has a theoretical oxygen binding capacity of 1.39 ml of oxygen per gram of hemoglobin, which is not reached physiologically. The real oxygen binding capacity of hemoglobin was determined by the German chemist Gustav Hüfner to be 1.34 ml of oxygen per gram of hemoglobin under normal conditions (von Hüfner 1889).

Another important effect for gas exchange is the Bohr effect. This describes the dependence of the oxygen binding capacity of hemoglobin in relation to the pH value or CO_2 partial pressure. Bohr et al. were able to show that at higher CO_2 partial pressures, hemoglobin binds less oxygen than at low CO_2 partial pressures (Bohr et al. 1904).

The lower pH favors the portioning of histidine residues in the hemoglobin structure, leading to stabilization of the t-form of hemoglobin, which explains the decreased oxygen binding affinity (Kovalevsky et al. 2010).

Organic phosphates, most notably 2,3-bisphosphoglycerate (2,3-BPG), have an effect on the oxygen affinity of hemoglobin (Benesch and Benesch 1967; Chanutin and Curnish 1967). 2,3-BPG binds to deoxyhemoglobin in a molar ratio of 1:1, thus favoring the t conformation. It has a stabilizing effect on the t-form, resulting in reduced oxygen affinity (Arnone 1972). 2,3-BPG binds differently to the different hemoglobin variants. An increase in 2,3-BPG concentration during pregnancy in fetal and maternal erythrocytes may also influence gas exchange across the placenta in favor of the fetus, since fetal hemoglobin has a lower affinity for 2,3-BPG (Delivoria-Papadopoulos et al. 1971).

The differences in the chain structure of hemoglobin are reflected in its oxygen binding properties. ◘ Table 4.1 gives an overview of the different physiologically occurring hemoglobin variants during human development.

Embryonic and fetal hemoglobin has a higher oxygen binding capacity than adult hemoglobin, which can be seen in the Hill coefficient and P_{50} oxygen saturation. Another difference between HbF and HbA is the difference in the Bohr effect. Oxygen

Table 4.1 Properties of the different hemoglobin variants in vitro

Hemoglobin	Hill Coefficient	P_{50} in vitro (mmHg)	P_{50} in vivo (mmHg)	2,3-BPG Binding Constant (mM)	Bohr Effect (Δlog P_{50}/ΔpH)	Reference
$\zeta_2\varepsilon_2$	1.7	1.4		0.09	−0.25	He and Russell (2001)
$\alpha_2\,\varepsilon_2$	2.3	2.7		0.17	−0.51	He and Russell (2001)
$_2\,\zeta\gamma_2$	2.3	5.9		6.0	−0.3	Hofmann et al. (1995)
$\zeta_2\beta_2$	1.6	1.9		0.30	−0.1	He and Russell (2001)
$\alpha_2\,\gamma_2$	2.65		20		−0.51	Zhang et al. (2003)
$\alpha_2\,\beta_2$	2.9	3.2 (9.8)	27	0.29 (0.45)	−0.54 (−0.41)	He and Russell (2001) and Hofmann et al. (1995)

binding is more reduced in HbA than in HbF when CO_2-partial pressure increases. This also favors oxygen transport from the mother to the fetus.

Compared to HbA, HbF has a low P_{50} oxygen saturation of 20 mmHg instead of 31 mmHg in the third trimester. This means that at a usual oxygen partial pressure of 30 mmHg in the umbilical vein, there is a 6-8% higher loading of fetal hemoglobin compared to maternal hemoglobin (Beer et al. 1958).

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Endocrinology of the Placenta

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Contents

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5.1 Introduction

The placenta is the largest endocrine organ during pregnancy dominating the entire hormonal system. Since the placenta is not innervated, all interaction with the mother as well as the fetus must be performed through hormonal signals. However, these "communication signals" secreted by the placenta into the maternal and fetal blood circulation can also act locally in the paracrine and autocrine regulation of placental function.

Placental peptide and steroid hormones influence all maternal endocrine functional circuits and regulatory axes. Even the not yet implanted embryo shortly after fertilization sends hormonal signals, especially human chorionic gonadotropin (hCG), which lead to the maintenance of the progesterone-producing corpus luteum in the ovary and its transformation into the corpus luteum graviditatis over the first ten weeks of pregnancy. At the same time, successful implantation into the uterine wall and early placentation are mediated in this way and via paracrine signals.

The hormones are synthesized mainly in the syncytiotrophoblast and the villous cytotrophoblast of the placental villi, but also in the extravillous trophoblast. These hormones can be subdivided according to their biochemical structure into (◘ Table 5.1):

- **Steroid hormones** are synthesized or modified in the trophoblast from maternal cholesterol, but also from fetal precursor hormones (◘ Fig. 5.1). Their synthesis thus embodies the concept of the fetoplacentomaternal unit established almost 50 years ago (Diczfalusy 1984). As lipophilic hormones, they are readily membrane-permeable and act at intracellular and nuclear receptors. However, membrane-bound receptors have also been described. In the following, the effects of progesterone, estrogen and glucocorticoids are highlighted.
- In addition to the placenta-specific hormones human chorionic gonadotropin (hCG), relaxin, human placental lactogen (hPL) and human placental growth hormone (hPGH), **peptide hormones** include a large number of other hormones and endocrine factors that are either identical to hormones occurring in endocrine homeostasis such as corticotrophin releasing hormone (CRH), leptin, gonadotropin releasing hormone (GnRH), etc. or are active at their receptors such as hCG at the gonadotropin receptors (Petraglia et al. 1996) (◘ Table 5.1).

Table 5.1 Overview of the most important placental hormones and their function. (Modified according to Costa 2016)

Hormone	Source	Receptor	Function	Cellular Regulation
Steroid hormones				
Progesterone	Syncytiotrophoblast	Nuclear receptor Membrane-associated receptor	Decidualization Embryo implantation Tranquilizing the myometrium Developing immunotolerance through – PIBF – ↑ Th2 cytokine production – ↓ uNK cell activity ↓ Trophoblast invasion ↑ Trophoblast migration ↓ Placental synthesis of hCG and leptin	PKA (↑) Estradiol (↑) Insulin (↑) IGF-1 (↑) Calcitriol (↑) Leptin (↓) CRH (↓) p38, ERK 1/2 (↑ 3βHSD)
Estradiol Estriol Estrone	Syncytiotrophoblast	Nuclear receptor Membrane-associated receptor	Endometrial receptivity and embryo implantation Angiogenesis and regulation of uteroplacental perfusion ↑ Myometrial contractility and induction of labor Growth of the mammary glands and preparation for lactation Syncytialization ↑ Placental leptin synthesis Hyperlipidemia and fat storage	cAMP (↑ aromatase) hCG (↑ aromatase) Estradiol (↑ aromatase) Cortisol (↑ aromatase) ERK 1/2 (↓ aromatase) CRH (↑) Leptin (↓) Insulin (↓)
Peptide Hormones				
hCG (human Chorionic Gonadotropin)	Syncytiotrophoblast	LH/hCG receptor	↑ Progesterone production in the corpus luteum graviditatis Syncytialization ↑ Decidual angiogenesis Support of immunotolerance ↑ Trophoblast invasion Tranquilization of the myometrium Embryo implantation	cAMP/PKA (↑) p38, ERK 1/2 (↑) PPAR-γ (↑) GnRH (↑) EGF (↑) Leptin (↑) Progesterone (↓) Activin (↑) Inhibin (↓)
hPL (human Placental Lactogen)	Syncytiotrophoblast Extravillous trophoblast	GH receptor Prolactin receptor	Lipolysis ↑ Free fatty acids and ketones ↑ Insulin sensitivity ↑ Fetal insulin and IGF-1 synthesis ↓ Placental leptin synthesis	cAMP (↑) GHRF (↑) Insulin (↑) EGF (↑) PPAR-γ (↑) Calcitriol (↑) Apolipoproteins (↑) IL-1, IL-6 (↑)

(continued)

Table 5.1 (continued)

Hormone	Source	Receptor	Function	Cellular Regulation
hPGH (human Placental Growth Hormone)	Syncytio-trophoblast Extravillous trophoblast	GH receptor	↑ Maternal IGF-1 synthesis ↑ Lipolysis Insulin resistance ↑ Blood glucose level ↑ Trophoblast invasion	cAMP (↑) Hypoglycemia (↑) Visfatin (↑) PPAR-γ (↑) Insulin (↓) Leptin (↓) Cortisol (↓)
Leptin	Syncytio-trophoblast Extravillous trophoblast	Leptin receptor	↑ Cytotrophoblast proliferation ↓ Cytotrophoblast apoptosis ↑ Trophoblast invasion ↑ hCG and ↓ hPGH synthesis ↓ Progesterone and estradiol production ↑ Angiogenesis Embryo implantation Uterine immunomodulation Tranquilization of the myometrium ↑ Proinflammatory cytokines and prostaglandins	cAMP/PKA (↑) PKC (↑) p38, ERK 1/2 (↑) hCG (↑) Estradiol (↑) Insulin (↑) Progesterone (↓) hPL (↓) Hypoxia (↓)
CRH (Corticotro-phin Releasing Hormone)	Syncytio-trophoblast	CRH receptor 1 and 2	↑ Remodeling by matrix metallo-proteinase 9 (MMP-9) ↑ Trophoblast invasion and growth Regulation of birth initiation Stimulation of prostaglandin effects by – ↑ 15-OH-prostaglandin dehydrogenase (PGDH) – ↑ Prostaglandin synthase (PGHS)-2 expression – ↓ Progesterone release Stimulation of maternal and fetal adrenergic regulation ↑ Cortisol and DHEAS concentration ↑ Placental estrogen synthesis	Neurotransmitter (NA, Ach) (↑) Neuropeptides – Angiotensin II (↑) – Vasopressin (↑) Interleukin-1 (↑) Glucocorticoids (↑) Progesterone (↓) NO (↓)
Adiponectin	Syncytio-trophoblast	Adiponectin receptor 1 and 2	↓ hCG, progesterone and hPL secretion ↓ Cytotrophoblast proliferation Syncytialization ↓ Placental insulin signaling ↑ Trophoblast invasion Modulation of uteroplacental angiogenesis ↑ Proinflammatory cytokines	Leptin (↓)? TNF-α (↓)? IL-6 (↓)?

Table 5.1 (continued)

Hormone	Source	Receptor	Function	Cellular Regulation
Activin A	Syncytio-trophoblast	Activin receptor IIa+b	↑ Cytotrophoblast proliferation ↑ Trophoblast invasion Decidualization and endometrial receptivity ↑ hCG and progesterone secretion ↑ Placental aromatase activity	Oxidative stress (↑) Proinflammatory cytokines (↑) CRH (↑) Hypoxia (↓)
Inhibin A	Syncytio-trophoblast	Activin receptor IIa+b	Antagonization of the Activin effects	hCG (↑) cAMP (↑) EGF (↑) GnRH (↑) Prostaglandin (↑) Activin (↓) Hypoxia (↓)

PKA Protein kinase A, *PKC* Protein kinase C, *PIBF* Progesterone-induced blocking factor, *IGF-1* Insulin-like growth factor 1, *ERK* Extracellular signal-regulating kinase, *cAMP* Cyclic adenosine monophosphate, *PPAR-y* Peroxisome proliferator activating receptor gamma, *GnRH* Gonadotropin releasing hormone, *EGF* Epidermal growth factor, *GHRF* Growth hormone releasing factor, *PHG* Placental growth hormone, *NO* Nitric oxide, *TNF-α* Tumor necrosis factor alpha.

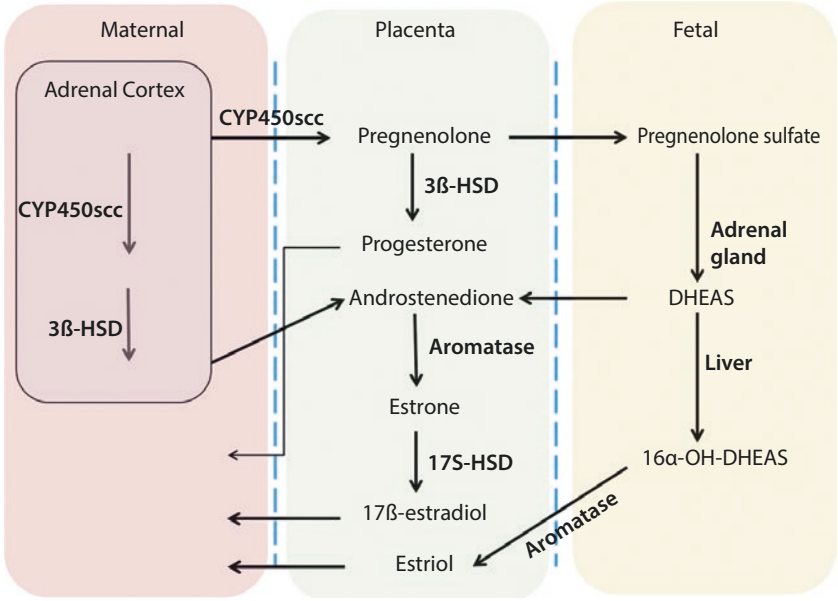

Fig. 5.1 Steroid synthesis in the feto-placento-maternal unit. *CYP450scc* cytochrome P450 "side-chain cleavage"; *3β-HSD* 3-Beta-hydroxysteroid dehydrogenase/Δ5-Δ4-isomerase, *17β-HSD* 17-Beta-hydroxysteroid dehydrogenase, *DHEAS* Dehydroepiandrosterone

5.2 Steroid Hormones

5.2.1 Progesterone

Progesterone is the central pregnancy-maintaining hormone. It is necessary for the relaxation of the myometrium, which enables a non-soft uterus free of contractions. At the same time it has anti-inflammatory and immunosuppressive functions, which enable the formation of the necessary immune tolerance at the fetomaternal interface.

Progesterone is initially formed in the corpus luteum graviditatis and, by decidualizing the endometrium, creates the prerequisite for nidation and implantation of the embryo. Both the maintenance of the corpus luteum graviditatis and its synthesis are controlled by hCG secreted by the conceptus and are thus the result of fetomaternal communication. After the 8th week of gestation, a luteoplacental shift takes place and the placenta takes over the synthesis of progesterone in sufficient quantity to maintain pregnancy (Tuckey 2005).

Progesterone is converted in the syncytium from maternal cholesterol by cytochrome P450scc ("side-chain-cleavage") to Δ5-pregnenolone and then to progesterone by means of the enzyme 3-beta-hydroxysteroid dehydrogenase/Δ5-Δ4-isomerase (3β-HSD) type I. The 3β-HSD activity is the limiting synthesis step in this process (Fig. 5.1).

Maternal blood progesterone levels increase during pregnancy until the last weeks before birth, but there is no decrease before the onset of labor in humans as in other species (Csapo et al. 1971).

Progesterone binds to both classical nuclear PRα and PRβ receptors, which are ubiquitously present throughout the genital tract and placenta, as well as other organs such as the mammary glands and brain. Upon binding, steroid receptors dimerize and exert their genomic effects in the nucleus by binding to specific promoter regions. In addition, PRβ can also activate cytoplasmic signaling pathways involved in proliferation, differentiation as well as angiogenesis and ultimately decidualization. Genomic progesterone effects are thus crucial for nidation and implantation of the embryo (Halasz and Szekeres-Bartho 2013). In addition, progesterone also mediates rapid, non-genomic effects through membrane-positioned receptors (MPRs) that decrease cAMP production through MAPK activation and regulate intracellular Ca^{2+} release (Goldman and Shalev 2007).

Progesterone is instrumental in the development of fetomaternal immune tolerance by enhancing the expression of Th2 cytokines and inhibiting the activity of uterine natural killer T cells (uNK). Their cytotoxicity is inhibited by progesterone-induced blocking factor (PIBF) (Halasz and Szekeres-Bartho 2013).

Inhibition of proinflammatory Th1 cytokines is also a mechanism for maintaining uterine tranquilization by decreasing prostaglandin and NF-κB synthesis. Progesterone also inhibits myometrial contractility through the expression of proteins essential for labor, such as matrix metalloproteases, Ca^{2+} channels, connexin 43 (a component of gap junctions relevant to labor synchrony), and oxytocin receptors (Kuon et al. 2015). Progesterone also acts as an antagonist of CRH-triggered induction of delivery.

5.2.2 Estrogens

Estrogens are synthesized in the ovary, the adrenal glands and the placenta. During the first weeks of pregnancy, estradiol synthesis is maintained in the corpus luteum graviditatis under the influence of hCG, before the syncytiothrophoblast takes over the production in large quantities, which increases over the entire course of pregnancy.

A group of estrogens is formed in the placenta: Estrone (E1), 17β-estradiol (E2), estriol (E3), and estrol (E4), but only E2 and E3 have clinical significance.

However, the placenta is unable to carry out the synthesis step of 17-hydroxylation of C21 steroids and therefore requires the precursors dehydroepiandrosterone sulfate (DHEAS) for estradiol and 16-hydroxy-DHEAS for estriol synthesis. DHEAS is utilized by both maternal and predominantly fetal adrenal cortex and is further aromatized to estrogens (◘ Fig. 5.1). Placental CYP450 aromatase activity is oxygen-dependent, so that estrogen production is decreased in placental insufficiency. In contrast, during fetal stress, elevated levels of estriol are found in maternal blood because its sole precursor, 16-OH-DHEAS, is derived from the activated fetal stress axis in such cases (Diczfalusy 1984). Before fetal status diagnosis by cardiotocography (CTG) and sonography was established, this was used to detect fetal compromise in antenatal care. Placental estrogen synthesis is lower in multiparous women and in pregnant women >30 years than in primigravidae and younger women (Toriola et al. 2011).

Estrogens act firstly via their nuclear receptors ERα and ERβ, which, like progesterone receptors dimerize after binding and modulate gene expression. ERα receptors are predominantly expressed in the cytotrophoblast, whereas ERβ receptors are more expressed in the syncytiotrophoblast. On the other hand, membrane-bound estrogen receptors exert nongenomic effects on e.g. activation of adenylate cyclase, intracellular Ca^{2+} mobilization and MAPK activity (Bjornstrom and Sjoberg 2005).

Estriol, which is derived exclusively from fetal precursors, is considered a weak estrogen but can be measured well in maternal urine. It has vasodilating effects on the uteroplacental vasculature and can thus optimize fetoplacental supply (Chang and Zhang 2008). At the same time, it increases gap junction formation in the myometrium via increased connexin-43 expression as a prerequisite for coordinated labor propagation across the uterus. This mechanism plays an essential role in the induction of labor at term as well as in threatened preterm birth (Di et al. 2001).

During pregnancy, however, **estradiol** plays a far more important role, both systemically and locally. Since it is secreted in large quantities into the maternal circulation, it plays an essential role in the adaptation of the maternal organism to pregnancy. Estrogen-induced changes in homeostasis during pregnancy lead, among others, to fluid retention and increase in blood volume, hypercoagulability, inhibition of lipolysis and hyperlipidemia, as well as to the growth of uterine and mammary gland tissues in preparation for lactation (Schleußner 2017).

Estradiol regulates uteroplacental angiogenesis and endothelial proliferation via ERα and ERβ receptors, as well as uterine and placental vasodilation via nongenomic effects (Corcoran et al. 2014). But even before that, estradiol supports embryo implantation by stimulating endometrial growth and differentiation, as well as cytotrophoblast differentiation and syncytialization via ERα (Groothuis et al. 2007; Bukovsky et al. 2003). At the end of pregnancy, estradiol, like estriol, induces the formation of gap junctions and, via ERα activation, the expression of oxytocin receptors in the myometrium, thereby increasing its contractility (Renthal et al. 2015).

5.2.3 Glucocorticoids

The placenta does not synthesize glucocorticoids de novo, but regulates fetal exposure through maternal stress hormones. Glucocorticoids play a central role in fetal organ development and maturation, so that the fetus must be protected from excessive maternal levels, which may have effects on the fetus such as fetal growth restriction and fetal (mis)programming with lifelong conse-

quences, e.g. for later hypertension or stress regulation (Schleußner 2016a).

The enzyme 11-beta-hydroxysteroid dehydrogenase (11ß-HSD), localized in the syncytiotrophoblast, catalyzes the reduction (11ß-HSD1) or oxidation (11ß-HSD2) of glucocorticoids. 11ß-HSD2 activity increases during pregnancy and converts active maternal cortisol to inactive cortisone, leaving the fetus unaffected by maternal cortisol levels. At term, the ratio of 11ß-HSD2:11ß-HSD1 activity increases, so that much more maternal cortisol enters the fetal circulation and contributes to the maturation of fetal adrenergic regulation (Wood and Keller-Wood 2016). In placental disorders with preeclampsia or fetal growth restriction, 11ß-HSD2 expression is reduced (Causevic and Mohaupt 2007).

Synthetic glucocorticoids such as beta- and dexamethasone or prednisolone are not or only slightly inactivated by 11ß-HSD, so that they have unrestricted effects on fetal stress regulation as well as fetal growth and maturation. This is used for the induction of fetal lung maturation with beta- or dexamethasone, but at the same time also leads to a significant placental and fetal growth restriction (Braun et al. 2015).

5.3 Peptide Hormones

5.3.1 Human Chorionic Gonadotropin (hCG)

hCG is the key essential embryonic hormone for the implantation of the embryo and the continuation of pregnancy. The embryo starts its hCG production already before implantation, so that hCG is detectable in the serum from day 8 post conception. During the first six weeks of gestation, embryonic hCG maintains the function of the maternal corpus luteum graviditatis and thus its progesterone and estrogen synthesis. Furthermore, it specifically induces decidual angiogenesis and later myometrial tranquilization. Through the activation of dentritic cells, uNK and regulatory T cells and the inhibition of cytotoxic T cells and their cytokines, it contributes significantly to local immune modulation and immune tolerance at the fetomaternal interface (Nwabuobi et al. 2017).

hCG is predominantly synthesized by the syncytiotrophoblast, but extravillous trophoblasts are also able to do so. The secretion, which mainly occurs into maternal blood, is pulsatile and doubles every 48 h until about week 10 of gestation, and then slowly decreases after this peak until the end of pregnancy (Costa 2016).

hCG belongs to the family of glycoproteins with a similar structure as the pituitary hormones LH (luteinizing hormone), FSH (follicle-stimulating hormone) and TSH (thyroid-stimulating hormone). All these hormones consist of two subunits that are noncovalently linked—with identical α-subunit, the β-subunit differs specifically, which is why ßhCG is always measured, e.g. as a pregnancy test. However, ßhCG is also 80–85% homologous to the LH ß-subunit. The ßhCG gene cluster is located on chromosome 19.

hCG activates adenylate cyclase, phospholipase C and ion channels, which in turn control intracellular cAMP, inositol phosphate and Ca^{2+} and regulate a variety of intracellular signaling cascades, such as e.g. protein kinase A and C, ERK1/2-MAPK or Smad2 through binding to the G-protein coupled LH/hCG gonadotropin receptor or through direct and indirect interaction with the TGFß receptor (Nwabuobi et al. 2017). cAMP, via protein kinase A, causes cytotrophoblast cell fusion and microvillous formation, which is essential for syncytiotrophoblast development.

At the same time, hCG/LH receptors are expressed in the cytotrophoblast, syncytiotrophoblast, and also the extravillous trophoblast, so that hCG also regulates trophoblast invasion and exerts local autocrine and paracrine effects (Handschuh et al. 2007).

hCG systemically alters maternal endocrine homeostasis. The pituitary-ovarian axis is suppressed by negative feedback regulation of hCG at the LH receptor of the adenohypophysis, resulting in ovarian dormancy during pregnancy (Schleußner 2017).

hCG also binds to the TSH receptors and thus stimulates thyroid function, whereas pituitary TSH secretion is slightly reduced in the first trimester of pregnancy. Excessively high hCG levels, e.g. in the case of a placental tumor (hydatidiform mole), can therefore trigger a full-blown thyrotoxic crisis. In the second half of pregnancy, TSH levels rise to maintain the physiological euthyroidism of pregnancy with intact pituitary-thyroid regulation. The essential mechanism for this is the doubling of estrogen-induced hepatic synthesis of thyroxine-binding globulin (TBG). Thus, with free thyroxine (T4) and triiodothyronine (T3) levels remaining constant, total T3 and T4 concentrations increase in the first half of pregnancy to remain stable after 20 weeks of gestation (Ross 2017).

5.3.2 Leptin

Leptin plays a central role in the regulation of energy homeostasis, appetite and body weight, but also influences angiogenesis, immune regulation and reproduction (Park and Ahima 2015). Mainly produced in adipocytes, the placenta synthesizes large amounts of leptin during pregnancy, which is secreted into the maternal and fetal circulation as well as into the amniotic fluid. During early pregnancy, leptin synthesis is highest in the syncytiotrophoblast. Maternal leptin levels increase throughout pregnancy until the beginning of the 3rd trimester and then remain stably high until birth (Costa 2016). With permanently elevated leptin levels, the mother is in a physiological leptin resistance, so that despite high leptin concentration, its central satiety effect is absent, resulting in hyperphagia and increased weight gain as well as hyperinsulinemia. This condition is accompanied by increased lipolysis, so that the mother can primarily use the lipids, which are only available to a limited extent in the placenta, as an energy source, while the glucose reserves are available for fetal supply. This can also be interpreted as a physiological adaptation to the increasing supply requirements of the growing fetus and as a mobilization of the maternal energy reserves (Ladyman et al. 2010).

At the same time, leptin plays an essential role in fetal growth control, angiogenesis and hematopoiesis. Within the placenta it has paracrine functions and has an immunomodulatory effect (Fig. 5.2) (Ashworth et al. 2000).

Leptin binds to the membranous leptin receptor (LepR), which is present ubiquitously in the human organism in six different splice variants and can activate JAK-STAT, ERK1/2 and PI3K signaling cascades. Leptin's paracrine effect is mediated by LepR in the syncytiotrophoblast, where it also influences the syncytial hormone synthesis, for example. It increases hCG production while inhibiting estradiol and progesterone synthesis (Costa 2016). In the extravillous trophoblast, leptin has a proliferative effect and is involved in the regulation of invasion via expression of metalloproteinases.

Leptin plays a key role in local immunomodulation by interfering with the regulation of prostaglandin and inflammatory cytokines, modulating uterine NK cell activity and stimulating trophoblast HLA-G expression (Pérez-Pérez et al. 2015). At the same time, it plays an important local angiogenic role in decidual neovascularization and uterine vascular remodeling by enhancing vascular endothelial growth factor (VEGF) expression.

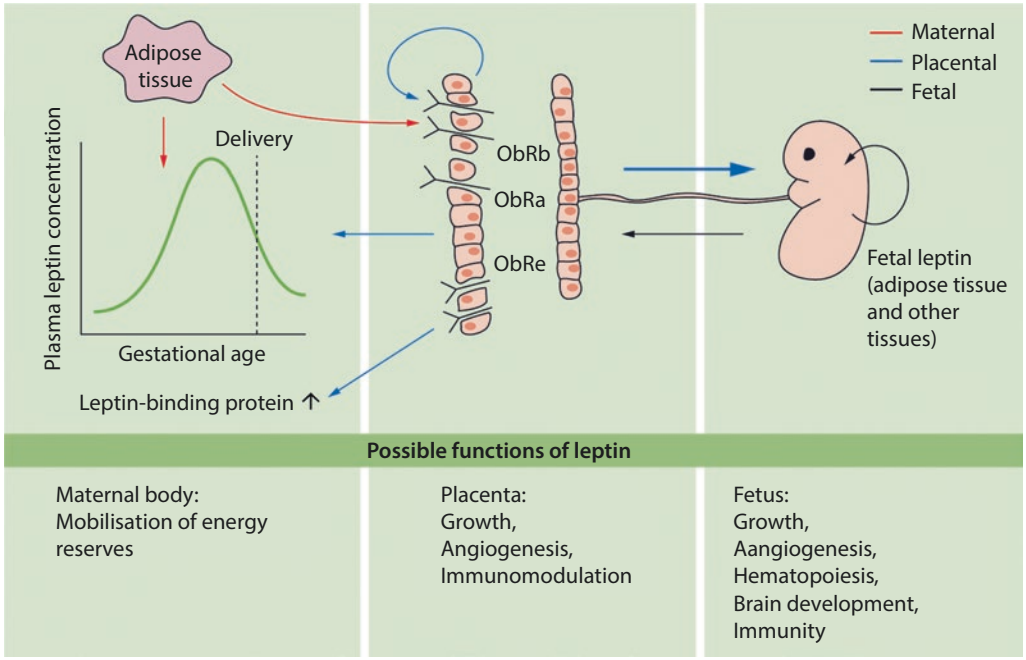

Fig. 5.2 Leptin as a placental regulator of maternal and fetal supply. (From Schleußner 2017, modified from Ashworth et al. 2000). *ObR* Obesity Receptor

5.3.3 Corticotrophin Releasing Hormone (CRH)

CRH is a 162 amino acid peptide hormone synthesized in the hypothalamus during pregnancy, but predominantly in the syncytiotrophoblast of the placenta and secreted into both the maternal and fetal circulation. Placental CRH plays a central role in physiological labor induction by coordinating fetal and maternal endocrine signals and activating the molecular mechanisms of labor induction (Fig. 5.3) (Schleußner 2016b).

CRH levels increase exponentially during pregnancy, and a correlation of concentrations already measured in the 1st trimester with the time of birth (preterm or term delivery, postterm pregnancy) was found. In preterm labor, significantly increased plasma levels are found compared to stable pregnancies of the same gestational age, (Petraglia et al. 2010).

Placental, as well as hypothalamic CRH synthesis, is modulated by neurotransmitters and neuropeptides such as vasopressin. In contrast to the regulation of the adrenergic hypothalamic–pituitary axis, placental CRH synthesis is subject to positive feedback from cortisol, which originates either from the fetal adrenal cortex or from the mother. This may also explain the labor-inducing effects of acute maternal or fetal stress. The administration of glucocorticoids for lung maturation also causes a transient increase in CRH, which can also lead to a temporary increase in uterine contractions (Schleussner et al. 2000).

Progesterone, on the other hand, inhibits trophoblast CRH synthesis. The antagonism of the two steroid hormones is of central importance in CRH regulation. While the

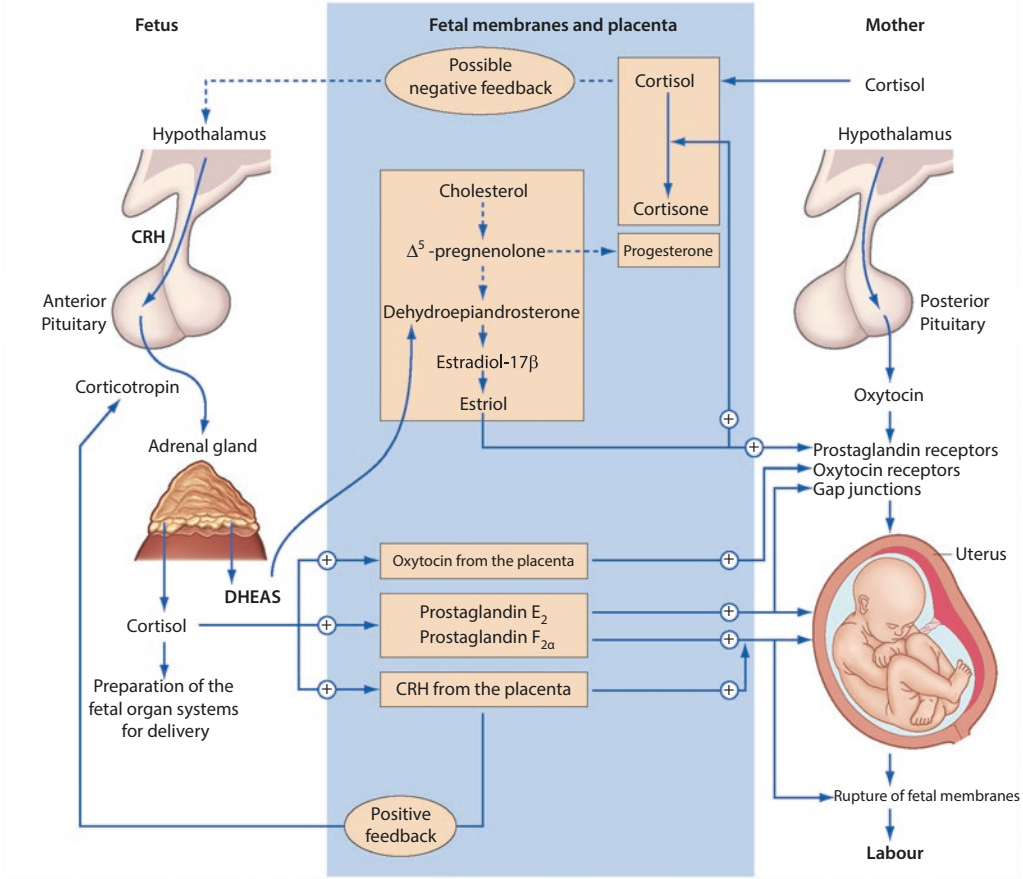

Fig. 5.3 Endocrine regulation of labor. (From Schleußner 2010, modified after Challis 2001). *CRH* Corticotrophin Releasing Hormone; *DHEAS* Dehydroepiandrosterone

inhibitory effect of progesterone predominates during pregnancy, increasing cortisol production by the fetal adrenal cortex towards the end of pregnancy leads to increased placental CRH secretion (Smith 2007).

The progressive CRH increase in turn leads to activation of the fetal adrenergic axis and thus also stimulates the secretion of fetal DHEAS, which is aromatized to estrogen in the placenta. As described in ▶ Sect. 5.2.2, this local estrogen dominance increases oxytocin receptor expression, gap junction formation and prostaglandin synthesis in the decidua, chorion and amnion. Prostaglandin synthesis enzymes (PGHS-2) are activated by increasing CRH levels, while at the same time the degradation of prostaglandin by prostaglandin dehydrogenases is inhibited. Thus, there is a direct effect of cortisol on both prostaglandin synthesis capacity and inhibition of prostaglandin metabolism (Challis 2001). Independently, direct effects of placental CRH on uterine contractility via myometrial CRH receptors are also found.

In addition to CRH, urocortins are also produced in the placenta, amnion and decidua, which belong to the CRH family and show up to 45% structural homology with CRH. Like CRH, urocortins act in stress regulation and birth induction, but also control endothelial vascular tone in uteroplacental perfusion (Petraglia et al. 2010).

5.3.4 Placental Lactogen (hPL) and Placental Growth Hormone (hPGH)

Human placental lactogen (hPL), as well as human placental growth hormone (hPGH) are peptide hormones, which show a large structural homology and are encoded in the same gene cluster on chromosome 17, whereby hPL functionally acts more like prolactin (Handwerger and Freemark 2000). Both hormones are synthesized mainly in the syncytiotrophoblast, but also in the extravillous trophoblast. hPL is detectable as early as two weeks post conception, hPGH from the 3rd week onwards. The levels of both hormones rise sharply over the course of pregnancy (hPL more than hPGH), while hypothalamic–pituitary GHRH and GH secretion are suppressed. Thus, placental lactogens and growth hormones take over control of both the maternal and fetal GH-IGF axes (Fig. 5.4) (Newbern and Freemark 2011).

Maternal hPL and hPGH blood levels correlate with placental size and are regulated by blood glucose concentration. Starvation and hypoglycemia stimulate hPGH secretion, while glucose administration inhibits hPGH secretion in vitro and in vivo. Unlike pituitary GH, hPGH is tonically secreted and not subject to control by GHRH, but is enhanced by PPAR-γ and cAMP and inhibited by leptin, insulin and cortisol. hPL, on the other hand, is stimulated by GHRH, insulin, apolipoproteins, PPAR-γ and cAMP (Costa 2016).

Both hormones bind to the ubiquitous membrane-bound GH receptor, although hPL has a low binding affinity, while it can also bind to the prolactin receptor. hPL enhances maternal lipolysis so that the resulting free fatty acids are available as an energy source. hPGH acts as an insulin antagonist and influences maternal glucose metabolism to ensure that maternal energy stores can be used for a constant supply to the fetus. It induces maternal IGF-1 synthesis, which contributes to genital organ growth. It also enhances uteroplacental perfusion and, in turn, fetoplacental supply. Thus, in a coordinated interplay of the placental hormones leptin, hPL and hPGh, maternal metabolism is tuned to the needs

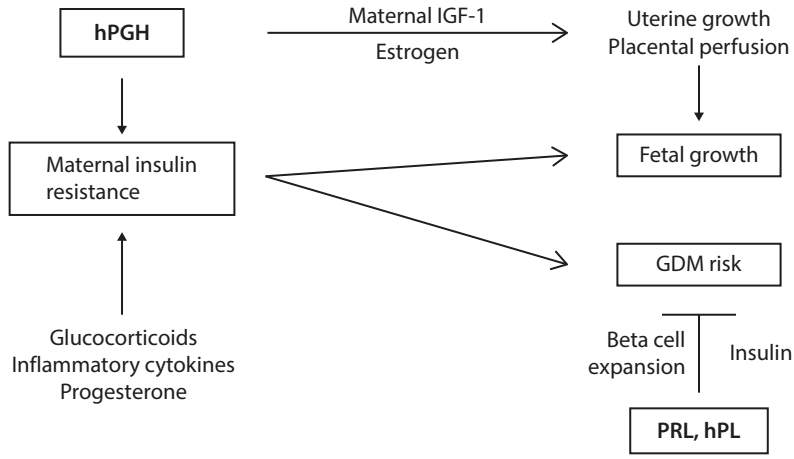

Fig. 5.4 Effects of placental lactogen (hPL) and placental growth hormone (hPGH) on the regulation of maternal metabolism and fetal growth and on the risk of gestational diabetes. (Modified from Newbern and Freemark 2011). *GDM* Gestational diabetes, *IGF-1* Insulin-like growth factor 1, *PRL* Prolactin

of fetal development (Fig. 5.4) (Newbern and Freemark 2011).

hPL is secreted into both fetal and maternal circulations, but hPGH is secreted only into maternal blood, so that its effects are directed exclusively to the maternal metabolism. hPL, on the other hand, directly affects fetal growth by enhancing IGF-1 synthesis in the fetal liver and pancreas.

Paracrine local effects of these hormones are still poorly understood, but since growth hormone receptors are also present in the placenta, these hormones have been demonstrated for placental growth and also trophoblast invasion.

A number of other growth factors are also secreted in the placenta, such as somatostatin, ghrelin, adiponectin, IGF-1 and IGF-2, which both regulate the GH-IGF axis and thus are the effectors of this axis (Petraglia et al. 1996).

5.3.5 Summary

The placenta is the largest endocrine organ during pregnancy dominating the entire hormonal system. Placental peptide and steroid hormones influence all maternal endocrine functional circuits and regulatory axes. Progesterone, as the central pregnancy-maintaining hormone, relaxes the myometrium and has anti-inflammatory and immunosuppressive functions that allow the formation of the necessary immune tolerance at the fetomaternal interface. Estrogens regulate maternal adaptation to pregnancy. Leptin, placental lactogen, and placental growth hormone alter maternal metabolism to use its resources to provide continuous fetal nutrition. Placental corticotrophin releasing hormone (CRH) is central to the initiation of labor as the placental clock.

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Teratology

Herbert Juch

Contents

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6.1 Introduction

Teratology is considered to be the science of "congenital anomalies", or congenital "malformations" or "birth defects". Originally, teratology was primarily morphologically oriented and a distinction was made in German between malformations and disruptions. In the case of malformations, an endogenous (genetic) cause was assumed for the congenital morphological peculiarities; in the case of disruptions, an exogenous cause was assumed as a disturbance of the development, which would otherwise have proceeded normally. In the meantime, the term "disruption" is no longer particularly common, and "malformation" is also frequently used for exogenously caused shape abnormalities.

In the teratological context, it makes sense to use the term "congenital anomaly" in order not to be limited to congenital abnormalities in the shape of the child, but also to include functional abnormalities. "Anomaly" is to be understood as a deviation from the norm, which has disease value, thus requiring therapeutic intervention. In Anglo-American usage, a distinction is often made between "major congenital anomalies" and "minor congenital anomalies", the latter being characterized by the fact that they do not represent functional or cosmetic impairments requiring medical treatment. In German, the term "große Fehlbildung" (major malformation) is occasionally used in reference to this. It should be noted that there are regular discussions in specialist circles about the classification of anomalies as "major" or "minor", as this classification can influence the assessment of teratological risks in the scientific literature.

Currently, the focus of teratological interest is not only on infantile organ or skeletal anomalies, but also on miscarriage and prematurity, placental dysfunction, intrauterine malnutrition and, in addition, prenatal influences and their possible long-term consequences for social and intelligence development or for disease dispositions.

Thus, a very broad field of activity has arisen for teratology in the meantime, motivated by the clear teratological mandate to recognize various exogenous negative influences on human prenatal development as such and to protect pregnant women from them as far as possible. In view of the wide range of tasks, it is hardly surprising that teratology today is more than ever an interdisciplinary science, with human genetics, gynecology and obstetrics, pediatrics, pharmacology and toxicology, epidemiology, hygiene and infectiology, occupational medicine and social medicine being particularly involved, in addition to embryology including placenta research, and each discipline has naturally developed its own approaches to the subject and its own focal points.

6.2 Congenital Anomalies Historically

Ever since there have been medical records, great attention has been paid to the morphological phenomenon of "miracle formations", from whose Greek term τέρας téras (miracle thing, omen, also monster) the term teratology is ultimately derived. The birth of a child with sometimes frightening, scary, mystically interpreted as "signs", was given great importance not only by the parents concerned, but also by society. There has always been a need to find the causes of these problems, which are experienced as fateful. In what we now consider "pre-scientific" times, concepts such as sin and guilt, as well as religious worldviews, played a central role in finding and combating the causes of congenital anomalies (cf. Schumacher et al. 1992). In part, however, quite useful explanations for observed health problems were already pos-

tulated in antiquity, such as the trauma theory, the spatial theory, or the disease theory (see the following overview). Nevertheless, not least Christian religious interpretations of the subject sometimes had life-threatening consequences for affected children, their parents, but also, objectively speaking, completely uninvolved third parties ("witches", "sorcerers", possibly "evil beggars").

Attempts to Explain Congenital Anomalies, Historically

- Trauma theory: e.g. blow to the abdomen of a pregnant woman
- Space theory: a too narrow uterus/too much semen
- Theory of disease, heredity: diseases of the fetus, familial clustering
- Lack of semen or "maternal matter"
- Rotten semen
- Woman's "Versehen": mother's imagination triggers malformations
- Poor posture of the pregnant woman
- Sins committed: e.g. "illegitimate" sexual intercourse
- Human-animal intercourse
- Devils, trolls, evil spirits
- Trickery of evil beggars (often people with congenital anomalies)
- God wants to show us his power

According to Schumacher et al. (1992) and Palister and Paré (1982).

The search for the causes of congenital anomalies has lost none of its explosive power to this day. The prenatal and postnatal medical-diagnostic effort expended for this purpose is now considerable. Nevertheless, in about two out of three affected children, no clear cause for the congenital health problems can be identified (Table 6.1).

Table 6.1 Causes of congenital anomalies, natural scientific

Causes	Examples	Share (%)
Genetic	Monogenic diseases, chromosomal anomalies	~11–30
Chemical/physical	Drugs, medication, pollutants, hyperthermia, hypoxia, ionizing radiation	~2–4
Maternal disease	Hypothyroidism, diabetes mellitus, gestoses, phenylketonuria, severe obesity, systemic lupus erythematosus (SLE), myasthenia, etc. **Infections:** Cytomegalovirus, rubella, Erythema infectiosum (Fifth disease), varicella, listeriosis, syphilis, toxoplasmosis, zika virus	~3
Anatomical	Twins, oligohydramnios, uterine anomalies	~3
Unclear	Polygenic or multifactorial anomalies, different combinations of endogenous and exogenous causes	~65–80

Adapted from Schäfer et al. (2012), Rösch and Steinbicker (2003), Schardein (2000), Enders (1991), and Wilson (1977)

6.3 Congenital Anomalies Today

Of course, the perception of congenital anomalies has significantly changed and objectified by the science-based, biopsychosocial approach. Today, congenital anomalies appear to us as a multi-layered problem, whereby various causally relevant levels hold important starting points both diagnostically and therapeutically (Fig. 6.1). The old question of guilt has, however, been preserved—albeit sometimes with a focus on the subconsciousness of those affected. It finds its contemporary expression, on the one hand, as a feeling of guilt and self-incrimination on the part of the parents concerned and, on the other hand, as an intensive legal search for guilt, e.g. on the part of medical doctors and midwives who accompany pregnancy and childbirth (which can also be clearly seen in their constantly rising insurance rates for the corresponding professional liability insurance policies).

Tip

The lack of a clear cause for the congenital anomalies of a child in about two out of three cases is perceived by most of the affected parents as particularly stressful and offers especially today, in the "post-factual" internet age, a lot of (virtual) space for various irrational to abstruse attempts at explanation. Therefore, a high degree of sensitivity, but also of clarity, is necessary from the professional side in the care of children with congenital anomalies and their parents, in order to avoid at least the errors and fallacies that are nowadays clearly identified as such.

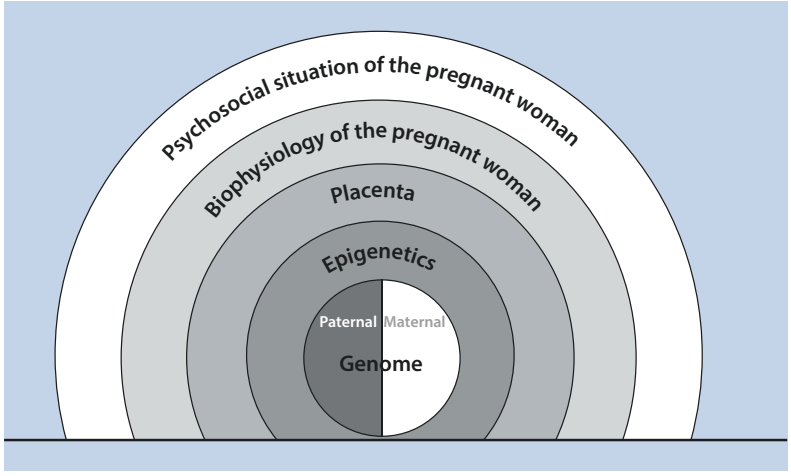

Fig. 6.1 Diagnostically and therapeutically relevant levels in connection with congenital anomalies. (Modified after Schumacher et al. 1992)

6.4 Basic Risk

Important for the understanding of congenital anomalies and their potential avoidability is the knowledge of a fundamental imperfection of all natural beings and developments—including the prenatal development of humans as embryos or fetuses. This finds expression in the so-called basic risk of congenital anomalies, i.e. a natural error rate or a lack of precision in our prenatal development. Even if, by all accounts, an ideal "healthy" pregnant woman (according to the WHO definition: "Health is a state of complete physical, mental and social well-being …") can be assumed to have an optimal course of pregnancy, without any family history of genetic diseases and without any recognizable external negative influences, congenital anomalies occasionally occur.

This baseline risk of congenital (i.e., not postnatally induced) health problems in humans in the 1st year of life is estimated to be ~3% for "normal" average healthy pregnant women, with normal average pregnancy outcomes. In addition, a ~15% "natural" risk of miscarriage is observed in the 1st trimester.

However, this risk of miscarriage increases markedly with increasing maternal age (and to a lesser extent with increasing paternal age) and thus indicates, among other things, a connection with the statistically more frequent age-related stochastic chromosomal abnormalities in oocytes (as well as in sperm cells). In addition, a certain proportion of these abortions can be attributed to pathological placentation (occasionally also immunologically caused), a direct indication of the placental influence on the basic reproductive risks.

These natural, undesirable events can sometimes be explained by random newly occurring dominant or familial recessive mutations in the germ cell genome. These events are also ultimately understandable in terms of systems theory and are due to the complexity of the system "prenatal human development" with its highly interconnected interactions of all genetic, epigenetic and exogenous factors. Similar to other highly complex systems, even with theoretical knowledge of all relevant measurands at the beginning of human development, the outcome of development could not be exactly predicted. Therefore, for embryonic and fetal development, sensitivity to developmental perturbations must be postulated through "weak causalities." Weak causality means, tiny changes in certain initial conditions, minimal fluctuations in physiologic parameters, which can lead to recognizable developmental differences, up to and including congenital anomalies, in the further course of development. In weather forecasting, this phenomenon is known as the "butterfly effect" (Lorenz 1972), where subtle air movements, as caused by butterflies, may be responsible for the development of a subsequent storm in a distant area. Different organ systems may well have different sensitivities in this respect, which would explain why the baseline risk for certain anomalies (e.g. heart malformations) is much higher than for others.

Various known risk factors increase these baseline reproductive risks to varying degrees, although usually no factor causes a 100% risk of abnormality.

From the concept of "baseline risk" it can be deduced that no pregnant woman has a guarantee of a healthy baby, at the same time, generally harmful influences will not lead to health problems in the baby with absolute certainty.

An important disadvantage of this statistically based approach, which reflects reality very well, is the impossibility of accurately predicting any congenital problems in individual cases. Prospectively, only probability statements can be made. In addition, retro-

spectively, if congenital anomalies are present in the child, the causality cannot be clarified exactly in many cases. For example, after a pregnant woman's one-time excessive alcohol consumption ("binge drinking") between the 4th and 8th week of embryonic development, an increased risk of congenital anomalies in the child is to be expected. The question of the concerned woman, whether the child will be damaged after this unintentional exposure to alcohol in early pregnancy or not, cannot be answered clearly with "yes" or "no". It could be stated that the chances of having a normal baby would probably be well over 90%. However, the chances would be statistically somewhat lower than they would have been without this incident. If, for example, a heart defect were to be diagnosed in the child after such an exposure, i.e. an anomaly whose basic risk is just under 1% and which we know is caused "multifactorially" (i.e. by various genetic factors in combination with various environmental factors), no clear causality of alcohol consumption could be established. However, a possible contribution of the undisputed teratogenic noxious agent alcohol could not be clearly excluded either.

6.5 Medication and Pregnancy

One aspect of teratology regularly receives special attention in the clinical field: the use of medication during pregnancy. This topic was strongly influenced by the thalidomide disaster at the end of the 1950s and beginning of the 1960s. Thalidomide, which was initially considered harmless and relatively non-toxic, turned out to be one of the most potent teratogenic substances in humans. Only after thousands of children had been born with sometimes severe limb malformations was it possible to identify thalidomide ingestion during pregnancy as the cause. As a consequence of this teratological worst case scenario, efforts in the field of reproductive toxicology were significantly intensified. For example, the insufficiency of reproductive toxicology testing of medications prior to marketing approval in only one animal species was recognized and mandatory teratological testing of agents in at least a second species was introduced. Teratological research on negative exogenous influences on prenatal development has generally received a strong boost in the wake of this medical scandal.

> As a negative after-effect of the thalidomide catastrophe, a partly undifferentiated and often exaggerated, frequently also completely unjustified concern about the use of medications during pregnancy has remained to this day.

This concern affects not only the pregnant women themselves, but not infrequently also their attending physicians. In line with the spirit of the times of an increasingly anxious society that is striving more and more for security, attempts are made in this area to "protect" themselves legally against possible lawsuits based on congenital anomalies in children by means of sophisticated non-binding formulations, and a primarily legal ethic is imposed on the medical doctor-patient relationship. The pregnant woman then easily gets the impression that she is not regarded as a responsible partner in the attempt to resolve a health problem, but as a client and potential opponent in court. This results in a loss of trust in the patient, and instead of facilitating a discerning risk-benefit assessment of a medication therapy during pregnancy, it occasionally leads to wrong decisions with negative long-term consequences.

Tip

To avoid such undeniably disadvantageous developments for both medical doctors and patients in the question of taking medication during pregnancy, the principle of "shared decision making" would be appropriate (Elwyn et al. 2012). This involves the patient to the maximum extent possible in the complex decision-making process.

Through optimal information about the opportunities and risks of the available therapeutic alternatives, the pregnant woman should be put in a position to decide on one of the possible therapeutic options (including "non-treatment") according to her individual needs and preferences, together with the attending physician. The prerequisite for such "informed decision-making" on the use of medication during pregnancy is, in addition to a realistic assessment of the performance of the therapeutic options, a realistic assessment of any teratological risks.

Tip

Support for individual risk assessment is provided by teratology information services (TISes, ► https://www.entis-org.eu; https://mothertobaby.org) set up worldwide.

These free-of-charge counselling facilities are also endeavoring to expand knowledge of teratogenic medication effects in humans by means of follow-up studies on a continuous and internationally networked basis. Through these activities, but also through various other teratological and pharmacoepidemiological studies, e.g. based on malformation and drug intake registries, a very comprehensive knowledge of the safety and risks of individual active substances in human pregnancy has been acquired over the past decades, which must of course be continuously expanded to enable a well-founded risk assessment in the future even for active substances that are currently little studied.

For individual teratological risk assessment, in addition to knowledge of the currently available data from observational studies on the use of specific medication in humans and of the available animal reprotox data, knowledge of the "basic rules" or "principles" of teratology established as early as the 1970s by James Wilson (Friedman 2010) has proved helpful (see following overview), which have only been slightly modified in the meantime. Based on fundamental findings on embryonic and fetal development from animal experiments and observations on human embryology, they facilitate both the individual optimization of therapy in the ideal case of therapy planning before the onset of pregnancy and the realistic risk assessment of accidental substance exposure in the case of unplanned pregnancy.

Wilson's Rules (Modified After Wilson 1977)

1. The sensitivity of the embryo to toxic influences depends on its genotype
   - Different medication effects in humans and experimental animals (species specificity of teratogenic effects)
   - Sensitivity to teratogens can vary from person to person (enzyme polymorphisms)
2. The sensitivity of the embryo to toxic influences depends on its developmental stage
   - Abortion or regeneration ("all or none" phenomenon) in the 1st and 2nd week of development
   - In the first trimester mostly severe morphological and functional anomalies (embryopathies)

- In the second and third trimesters, mostly mild, predominantly functional anomalies (fetopathies)
- Specific teratogenic noxious agents often only have an effect at certain times in development

3. Different embryotoxic influences affect (embryonic) development via relatively few specific mechanisms
   - Spectrum of possible congenital anomalies is limited
   - Various harmful influences or genetic causes cause similar abnormalities
   - A teratogenic effect does not increase the risk of all possible congenital anomalies
4. After exposure to teratogens, the following developmental courses are possible in principle
   - Normal development, defects are completely repaired (especially 1st and 2nd week of development)
   - Intrauterine fetal death, miscarriage, premature birth
   - Morphological organ anomalies, functional limitations, growth restriction
   - Tumor development
5. The way toxic influences reach the embryo/fetus depends on their physical and chemical properties
   - Pharmacokinetics in pregnancy:
     - Medication kinetics in the mother
     - Medication kinetics in placenta/embryo/fetus
6. In teratology, dose-response relationships apply as elsewhere in pharmacology and toxicology
   - Minimal teratogenic dose thresholds for teratogenic effects (NOAEL, "no observed adverse effect level")
   - Lower dose, lower risk of damage with exposure above threshold

6.6 Placenta and Teratology

The placenta plays a central role, especially in the context of exogenously influenced and co-induced congenital anomalies, on the one hand due to its complex barrier function for diverse exogenous influences during prenatal development and on the other hand as a direct target organ for teratogenic damage with consecutive placental pathologies (► Chaps. 7, 8, 10, 11) and secondary pregnancy complications as well as health problems in the child.

To shed light on the role of the placenta from the point of view of teratology, an attempt will now be made to illustrate its significance on the basis of the basic teratological rules.

6.6.1 The Sensitivity of the Embryo to Toxic Influences Depends on the Genotype

A classical derivation from this basic rule refers to the limited applicability of reproductive toxicological data obtained in animal experiments to the human situation.

The fundamental species differences in the genetic blueprint of mammals are particularly evident phenotypically in the very different strategies of placentation.

Macroscopically and tissue architecturally, interesting variations can be observed in this regard. From a functional point of view, both with regard to the organization of the perfusion of the organ and with regard to the invasive behavior of the placenta and the associated different challenges for the maternal immune system (tolerance, invasion control), there are major differences between

species. In terms of embryonic toxicology, these genetically determined differences are of particular importance when attempting to apply the correlations between blood levels of potentially harmful substances and their concentration in the embryonic and fetal compartments, which have been established in common animal models (mouse, rat, rabbit), to the situation in humans. In the case of readily absorbable lipophilic small molecules, which also efficiently cross other tissue barriers such as the blood-brain barrier and are distributed more or less over all body compartments anyway, smaller species differences in embryonic or fetal exposure are more likely to be expected. In contrast, this may be much more differentiated in the case of larger molecules, such as the biologicals that are already being used more and more frequently (see below), or nanoparticles that may be used in the future for diagnostic or therapeutic purposes.

In any case, genetically determined species-specific differences in the metabolism of the tissues involved in the placental barrier, in particular the trophoblast cell layers, but also the endothelial cells of the fetal vessels, must be taken into account for lipophilic small molecules. Depending on their transporter and enzyme equipment or activity, the exposure of the embryo or fetus may vary considerably at a given maternal blood level (see ► Sect. 6.6.5 "The way in which toxic influences reach the embryo/fetus depends on their physical and chemical properties").

However, this first basic rule also points to possible differences in sensitivity to teratogenic noxae **within** a species, which can be observed even with absolutely identical exposure due to identical maternal blood levels. This was impressively demonstrated in a dizygotic and heteropaternal pregnancy (two oocytes, two different fathers) of an epilepsy patient who had been treated with phenytoin (Phelan et al. 1982). One twin was diagnosed with a "fetal hydantoin syndrome" with multiple congenital anomalies, while the second child was completely normal. Genetically different epoxide hydroxylase (EPHX1) enzyme activity was identified as the presumptive cause. The affected child showed low activity of this enzyme responsible for the degradation of toxic phenytoin metabolites, while the unaffected child showed high activity. EPHX1 appears to be expressed to a greater or lesser extent by almost all cells in the body however, there is currently no knowledge of the extent of expression in the early placenta, so that a possible placental contribution to the described discordance of the twin children cannot be estimated.

Nevertheless, initial work on a defined placenta-specific pharmacogenetic contribution to fetal exposure is already available, e.g. in the context of known genetic variants of transport proteins from the group of multi-drug resistance proteins such as ABCB1 (Bliek et al. 2009). The plausible background for these considerations is that certain alleles of such transporters are associated with a reduced function of these "detoxification molecules" expressed in the placenta. Expectant mothers (and fathers) who can pass on such genetic variants with reduced activity to their offspring would have a higher risk of congenital anomalies in the child in the event of foreign substance (e.g. medication) exposure during pregnancy. However, it would be important to know the actual genotype of the child during pregnancy. At present, this could only be reliably analyzed by means of invasive prenatal diagnostics (increased risk of miscarriage due to procedures such as amniocentesis or chorionic villus sampling). Against the background of the rapid developments in the field of the analysis of fetal DNA from maternal blood, an analysis of relevant fetal genetic

characteristics with known influence on placental medication kinetics could perhaps be carried out in the future without increasing the miscarriage risk. This would then allow an individually optimal medication selection to be made with the greatest possible safety with regard to fetal exposure.

6.6.2 The Sensitivity of the Embryo to Toxic Influences Depends on Its Stage of Development

The striking (histo-)morphological and functional changes of the human placenta, especially of the placental barrier, in the course of pregnancy also contribute to the observed temporarily different sensitivity to exogenous influences.

This change is particularly evident when looking at the exposure of the embryo or fetus to antibody molecules in maternal blood. In the first trimester it is assumed that also immunoglobulins of the IgG class (natural "nanoparticles" with a diameter of ~10 nm) do not cross the consistently double-layered villous trophoblast (► Chap. 1), but only accumulate in the syncytium. An active physiological transfer of maternal IgG to the fetus only begins in the second trimester. In the third trimester, this transfer develops into a very efficient passive immunization of the fetus to provide for the fetal "nest protection" in the first three months of life (Palmeira et al. 2012).

This circumstance has been relevant in fetal-maternal medicine up to now, for example, in connection with the now rare problem of "Rh incompatibility" in Rh-negative mothers and Rh-positive fetuses. This can result in varying degrees of fetal hemolysis due to maternal anti-rhesus IgG antibodies (mostly anti-D antibodies). This "morbus haemolyticus" develops only after the 18th week of pregnancy, especially if the routine "rhesus prophylaxis" to prevent "immunization" of the mother with fetal erythrocytes was not (successfully) carried out in a previous Rh-positive pregnancy. Otherwise, maternal immunoglobulins still play a pathophysiological role in rare autoimmunological diseases of the pregnant woman with pathological antibody production.

In systemic lupus erythematosus (SLE), damage to conduction in the fetal heart (atrioventricular block, AV block) by certain maternal Ro/La IgG occasionally occurs from the 16th week of pregnancy onwards, as well as not infrequently to (transient, harmless) neonatal skin manifestations in the sense of "neonatal lupus". Also in myasthenia gravis, where in the more frequent and harmless case a transient neonatal muscle weakness occurs, severe intrauterine movement disorders of the fetus and consequently permanent limb deformities in the child ("arthrogryposis multiplex congenita") are sometimes found due to antibodies against the fetal motor endplates. A rare neonatal Graves' disease, a transient hyperthyroidism caused by autoantibodies (possibly with additional eye problems), has also been described in newborns of affected mothers.

In the meantime, an interesting additional aspect has emerged from a medical point of view. Many medications from the group of "biologicals" are recombinantly produced monoclonal antibodies of the IgG class, which are mainly used in oncology, but increasingly also in rheumatic and immunological diseases. The increased use of these very specifically effective medications in women in the fertile phase of life repeatedly leads to unintentional exposure in early pregnancy, especially since experience shows that 30–50% of pregnancies occur unplanned. From a placentological point of view, practically no placental transfer of these therapeutic antibody molecules would be expected in the first trimester and thus in

the particularly sensitive embryonic development phase. Hence, the teratogenic risk of exposure in early pregnancy would theoretically be classified as very low, unless the antibody causes direct placental damage. However, if the treatment is continued into the second and third trimester, an increasing fetal exposure and, depending on the specific target molecule of the therapeutic antibody, possible adverse effects on the fetus would have to be expected.

These considerations should also take into account the placenta itself (or its immunological situation) as a possible site of (side) effects of biologicals.

Therefore, in the case of very early exposure to biologicals, the question of a possible increased rate of cardiac malformation arises less than the question of a possible placental pathology in the sense of an increased tendency to miscarriage or an increased risk of FGR ("fetal growth restriction"), preeclampsia or premature birth. From this perspective, a pharmacoepidemiological study from 2009, which, e.g., links the use of TNF-alpha inhibitors on an IgG basis with an increased risk of embryopathies, should be critically discussed (Carter et al. 2009). However, it should not go unmentioned here that in analyses of the fluid from the chorionic cavity of early human developmental stages, contrary to the current notion of immunoglobulin transfer in the human placenta, immunoglobulins were also detected, although it remains completely unclear how these can enter this compartment through the early placenta (Gulbis et al. 1992).

Ultimately, only the consistent follow-up analysis of a sufficiently large number of children prenatally exposed to biologicals, in comparison to unexposed children of an as adequate as possible comparison cohort, will be able to clarify which human teratogenic risks such biologicals actually entail, depending on the application during pregnancy (e.g. Weber-Schoendorfer et al. 2015). It would also be desirable that in future these studies take greater account of the possible placenta-associated consequences on prenatal development, which currently receive little attention in research. Interesting in this context is, for example, the observation of a possible benefit of anti-inflammatory TNF-alpha inhibitor therapy in habitual abortions (Winger and Reed 2008).

Further evidence of the placental time dependence of teratogenic effects is found in the differential fetal infection rate of transplacentally transmitted infections during pregnancy, depending on the time of infection. This phenomenon can be considered separately from the differences in potential fetal harm from such intrauterine teratogenic infections, depending on their occurrence in relation to embryonic or fetal development.

Toxoplasmosis may be mentioned as an example. In the case of a primary infection with Toxoplasma gondii during pregnancy, an infection of the offspring is observed in ~50% on average, whereby after infection in the first trimester the fetal infection rate is reported to be 15–25%, in the second trimester ~54% and in the third trimester ~65%. Before the 16th week of gestation, infection of the fetus does not seem possible even with maternal initial infection in the first trimester, due to the placental barrier. Nevertheless, the rarer early infection leads to more severe congenital anomalies in the fetus than the later one (Enders 1991). Similarly, primary cytomegalovirus infections in pregnancy are expected to cause severe damage of the child in ~50% of infected embryos or fetuses when infected in the first trimester, with a transmission rate of ~20%. In the case of maternal infection in the third trimester, placental transmission is found in >80% of cases, but with a significantly lower risk of fetal damage (see RKI Guide for Physicians 2014).

Interesting in this context is also the dependence of a severe neonatal varicella disease on the existing possibility of "passive vaccination of the fetus" through the placenta. If infection occurs sufficiently long before delivery, anti-varicella IgG produced by the maternal immune system can be transferred across the placenta in efficient amounts, thus preventing severe neonatal disease. The same applies to measles infection and mumps infection during pregnancy, which only lead to severe neonatal disease when primary infection occurs shortly before birth (Enders 1991).

The consequence of teratogenic damage to the placenta itself is in principle also dependent on the time of exposure in the course of pregnancy. Thus, in the case of damage in the first trimester, a reduced vascular remodeling of the spiral arteries with subsequent development of a typical growth restricted, thickened and, at the latest in the third trimester, functionally insufficient FGR placenta (▶ Chap. 11) would be possible. Although the same damaging influence in the third trimester would possibly result in a functional disturbance of the placenta or necrosis and thus a supply disturbance of the fetus, the placental morphology would not be impaired in the same way as in the case of early exposure.

6.6.3 Different Embryotoxic Influences Affect (Embryonic) Development via Relatively Few Specific Mechanisms

The most obvious example of the restricted response variability to exogenous damage is the "all-or-nothing principle" of teratogenic exposure in the 1st and 2nd week of development.

Due to the low specialization of the cells in the early embryo, any damage can be completely repaired, so that no congenital anomalies are to be expected as a consequence. Alternatively, if the damage is sufficiently severe (not least in the placental tissue, which clearly predominates in terms of quantity in this phase of development!), a clinically inconspicuous early abortion occurs. There is only a normal onset of menstruation at the end of the 2nd week of development (corresponding to the 4th week of pregnancy calculated after the first day of the last menstrual period) at the expected time. In any case, regardless of the nature of the harmful influence (mitotic poison, ionizing radiation in the appropriate dose, infection, etc.), the consequence would be identical. More differentiated, but still limited, are the pathomorphological expressions of the placenta (▶ Chaps. 8, 10, 11) in the case of damage from the 3rd week of development until birth.

With regard to pathomechanisms of placental damage that have been completely deciphered in molecular detail, it is true, as for pathomechanisms of congenital anomalies in general, that we are far from knowing all of the theoretically limited mechanisms that exist. Often, our current knowledge about exogenously, but also endogenously caused developmental anomalies is based only on empirical data.

6.6.4 After Exposure to Teratogens, Different Developmental Courses Are Possible in Principle

The fourth basic rule describes the fact that exposure to a potentially teratogenic factor alone does not necessarily lead to congenital health problems. This can be deduced conclusively from the other rules (dependence

of an effect on individual genetics, the period of exposure, the dose, etc.) and is not least also due to placental phenomena. In addition, however, this rule not only focuses on malformations in the embryo or fetus, but also expands the focus to additional, more placenta-associated pregnancy problems (miscarriage, prematurity, FGR, etc.). These problems are explicitly considered as a consequence of teratogenic exposure, and the placenta is thus identified not only as a mediator of teratogenic effects, but also as a target organ of such damage. It should be critically noted at this point that research on the use of medication during pregnancy and on the teratogenic effects possibly caused by them regularly examines, in addition to any congenital anomalies, the miscarriage rates and the duration of pregnancy. However, these studies usually do not collect data on the simplest placenta parameters such as placental weight. Likewise, these examinations usually lack ultrasound checks of fetal growth to detect any slowing of intrauterine growth especially in fetuses whose birth weight is not below the 10th percentile, but in whom a bend in the growth curve would indicate FGR and be detectable in this way.

6.6.5 The Way in Which Toxic Influences Reach the Embryo/Fetus Depends on Their Physical and Chemical Properties

This rule refers directly to pharmacokinetics at the placental barrier. Only a few teratogenic influences, such as ionizing radiation, whose negative influence also depends on the physical properties of the radiation (alpha, beta, gamma rays, other particle rays, ionization density, wavelength, dose), or possible ascending infections from the vagina, which can also reach the amniotic fluid directly via the fetal membranes, bypassing the placenta, are **not** influenced by the physicochemical interaction with the placental barrier in their effect on embryonic or fetal development.

Little is known about the efficiency of substance transfer in the early human placenta or in the second trimester. The situation of the mature placenta in the third trimester, on the other hand, can be simulated comparatively well in this respect (► Chap. 15) or determined in vivo (by comparing the maternal blood level with the fetal umbilical cord blood level measured immediately after birth).

In general, a molecular mass <600 D and lipophilicity are beneficial for placental passage. Most classical, orally applicable medications and all addictive drugs fall into this category, so that infantile "co-treatment" cannot usually be ruled out.

However, the physico-chemical barrier function of the placenta occasionally allows a therapy restricted purely to the mother, e.g. with insulin (5800 D) in diabetic women, but also an anticoagulant therapy of the pregnant woman with low molecular weight heparin (~5000 D). Similarly, new high-molecular-weight agents such as Abciximab, Ranibizumab or Certolizumab, which consist only of (modified) Fab fragments of monoclonal antibodies (min. 50,000 D), or Anakinra, an interleukin-1 receptor antagonist (>17,000 D), should be expected to have a comparatively very low infant "co-treatment". Likewise, fibrinolysis by means of alteplase (molecular mass of >59,000 D) should be limited mainly to the maternal circulation.

Active placental transport mechanisms, which can ensure that large hydrophilic molecules with a mass of 150,000 D are efficiently transported from the maternal blood into the fetal circulation, play an important role for the placental barrier. This can be seen in the example of IgG transport in the

second and third trimesters, where the IgG Fc part is specifically channeled through the syncytium via a neonatal Fc receptor (FcRn) and receptor-mediated transcytosis (point 2 "The sensitivity of the embryo to toxic influences depends on its developmental stage").

Besides specific transport mechanisms for very large molecules, placental metabolism as well as transport proteins from the group of multi-drug resistance proteins have to be considered.

Multi-drug resistance proteins (specifically BCRP, "breast cancer resistance protein") ensure that e.g. the blood sugar-lowering substance glibenclamide is not transferred in significant quantities across the placenta despite good oral bioavailability and small molecular weight, in contrast to other similar active substances from the sulfonylurea group. The agent is exported quite efficiently by active transport, from the trophoblast into the maternal circulation via BCRP molecules. In addition, high plasma protein binding and a short half-life of glibenclamide in the blood also contribute to the fact that the drug does not transfer appreciably into the fetal circulation and is discussed as a possible alternative to insulin therapy in type 2 diabetes or gestational diabetes in pregnancy.

The difference in placental inactivation between halogenated glucocorticoids such as betamethasone and dexamethasone on the one hand and non-halogenated ones such as cortisol or prednisolone on the other hand is responsible for the fact that only about 10% of a maternal prednisolone concentration is measured in the umbilical cord blood, but about 30% of a beta- and almost 100% of a dexamethasone concentration (Schäfer et al. 2012). This finding can be used to prioritize prednisolone when maternal therapy is needed during pregnancy. In contrast, dexamethasone would be the medication of choice for infant glucocorticoid requirements, e.g. to reduce increased androgen production in the adrenal gland of female fetuses and thus to prevent virilization of the external genitalia in cases of prenatally diagnosed adrenogenital syndrome (AGS).

6.6.6 Dose-response Relationships Apply in Teratology as Elsewhere in Pharmacology and Toxicology

The old basic rule of toxicology "the dose makes the poison" is of course also valid for placental as well as for embryonic or fetal exogenously caused damage. Any exposure, regardless of the substance, can be toxic and thus teratogenic, or non-toxic, depending on the dose. Figure 6.2 illustrates this basic principle.

The placenta usually plays a key role for the embryonic or fetal dose, in addition to the fetal liver metabolism and a possible substance accumulation in the amniotic fluid. As previously discussed in the context of the other Wilson's rules, the fetal dose does not depend exclusively on the maternal blood level.

Placental genetics as well as developmental placental structure and physiology and the particular species-specific placental barrier function significantly influence the fetal dose and thus ultimately a teratogenic effect.

In the case of a teratogenic effect, a threshold dose (NOAEL, "no observable adverse effect level") must be assumed, above which teratogenic effects become apparent, depending on the genetically determined individual sensitivity. Below this "minimum dose", the risk of congenital anomalies is not increased. Unfortunately, NOAEL values for most substance exposures are still unknown. This is also due to the fact that in

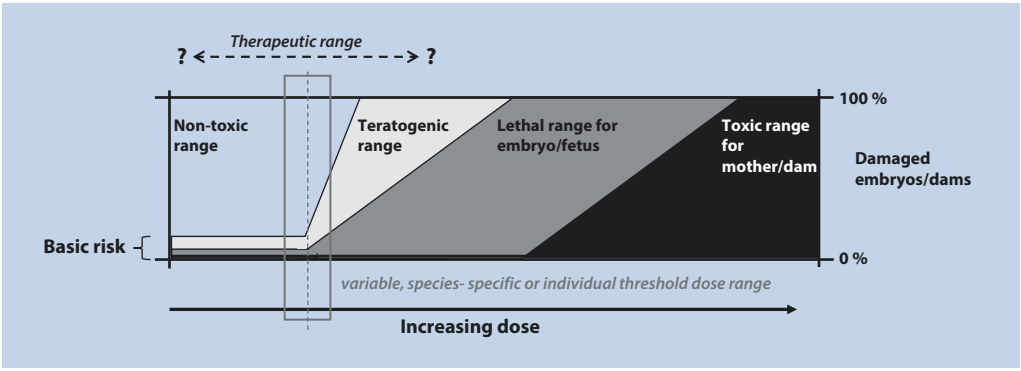

Fig. 6.2 Dose-response relationships in teratology determined by animal experiments. (Modified after Wilson 1977 and Schäfer et al. 2012)

most observational studies of medication exposure during pregnancy, although the amount of medication taken is recorded, maternal drug blood levels are not determined. Hence, no clear correlation can be established between teratogenic effects and drug dose. This would only be possible if the individual maternal pharmacokinetics were taken into account, as well as the often noticeably reduced tendency during pregnancy to actually take the prescribed amount of medication (Lupattelli et al. 2014). Both could be easily objectified on the basis of measured medication levels in the blood, to effectively document at least the placental substance load. For a few drugs that show a significantly altered metabolism and excretion due to pregnancy, the recommendation to regularly monitor serum levels during pregnancy in the sense of "therapeutic drug monitoring" (TDM) has become established to avoid under- or overdosing. Therapy with the antiepileptic drug lamotrigine or treatment with lithium would be examples of this (Schäfer et al. 2012).

Currently, a new dimension is emerging in teratology. The question is increasingly being asked as to which prenatal influences affect the long-term health of the child in later life, i.e. the tendency to high blood pressure, obesity, diabetes, arteriosclerosis or cancer, psychosocial and intelligence development, the predisposition to psychiatric diseases, immunological development and the ageing process. A theoretical basis for these considerations is provided by the Barker or DOHaD (Developmental Origins of Health and Disease) hypothesis (Wadhwa et al. 2009). This hypothesis initially drew attention to the possible influence of factors during prenatal development on later disease risks, based on epidemiological observations of associations between maternal nutritional problems in pregnancy and the risk of cardiovascular disease in children decades later. Epigenetic changes are under discussion as important pathomechanisms responsible for these observations, which, given a genetic background, allow for sustained exogenously induced modulability, so that prenatally induced long-term functional maladaptations and disorders appear possible. Such epigenetically mediated phenomena could also be caused as early as the all-or-nothing phase, as discussed in connection with teratogenic effects and epigenetic changes caused by in vitro fertilization (IVF) methods (Wilkins-Haug 2009).

6.7 Conclusion

In summary, it can be stated that in the old teratological question of the causes of congenital health problems in a variety of ways, the placenta plays a relevant role in answering it. This multifaceted involvement of the placenta in teratology is in part already quite clearly definable, and an understanding of its importance is now medically applicable. In many areas, however, there is obviously still a great need for research, and we can certainly expect further diagnostically or therapeutically useful placentological findings in teratology in the future.

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The Effects of Legal and Illegal Drugs on Placental Function

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Contents

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7.1 Introduction

According to the Ministry of Health, 1,800,000 people in Germany were dependent on alcohol in 2015, and an estimated 2,300,000 people were dependent on medication. Around 600,000 people display a problematic use of cannabis and other illegal drugs. Most recently, data were published in February 2017, according to which 2,650,000 children in Germany live in families with addiction problems, and of these, 60,000 live in families with illegal drug use, although it is assumed that the number of unreported cases is significantly higher (Moesgen et al. 2017).

For most pregnant women, pregnancy is a special phase of life in which they responsibly decide to do everything possible to ensure the optimal development of the unborn child, in particular by stopping smoking and drinking alcohol or abstaining from drug use. For the vast majority, any drug use is obsolete, but for an unfortunately not diminishing minority, this is not the case. Reliable German data do not exist, for the USA a constant figure of 5.9% pregnant women with illegal drug use is reported (Ross et al. 2015).

Prenatal drug exposure poses a variety of risks to mother and child that can lead to obstetric and neonatal complications. The most common problems arise due to disturbances in placental function: fetal growth restriction (FGR), preeclampsia, prematurity, premature rupture of membranes (PROM) to premature placental abruption and intrauterine fetal death (IUFD).

The placenta-associated effects of the major licit and illicit drugs and possible pathomechanisms in the placenta are described below.

7.2 Smoking During Pregnancy

The WHO estimates that smoking during pregnancy is the most important preventable risk factor for a number of pregnancy complications. While FGR, preterm birth and stillbirth are significantly more common, preeclampsia occurs less frequently in pregnant women who smoke.

Although overall tobacco use, particularly during pregnancy, has declined in most developed countries in recent decades, prevalence is still thought to be 10–20%. Pregnant women who smoke are often younger, single or have a partner who also smokes, have unplanned pregnancies, are not employed and have a lower socioeconomic and educational status. Interestingly, smoking is less prevalent among women of reproductive age with a migration background (German Ministry of Health [Bundesministerium für Gesundheit] 2016).

Through targeted intervention at the beginning of pregnancy, it is realistic to reduce the number of smokers to about half. The most promising results were achieved in studies that offered counselling and problem-solving strategies and in those that used incentive measures (e.g. vouchers for special additional services) and personal feedback (ultrasound monitoring of normal child development, determination of cotinine levels in urine as a nicotine breakdown product and thus direct evidence of nicotine consumption). In its recommendations, the German Medical Association has adopted the so-called 5A scheme of the WHO (Ask-Advise-Assess-Assist-Arrange), according to which tobacco consumption can and should be addressed at the beginning of pregnancy. Non-individualized health campaigns tended to be less successful. Meta-analyses also demonstrate that

about half of those women who temporarily abstained from tobacco smoking during pregnancy resume smoking within the first 6 weeks to 6 months after pregnancy.

7.2.1 Tobacco Ingredients

Tobacco consists of a mixture of chemical substances, of which for nicotine, carbon monoxide CO, cadmium and benzopyrene the effect on the placenta have been studied.

Nicotine crosses the placental barrier unhindered and, in the case of chronic abuse, reaches the same levels in the fetal blood as in the mother (Lambers and Clark 1996). Nicotinic receptors are found in almost all placental cells, including the syncytiotrophoblast, the Hofbauer cells, the endothelium and the vascular musculature (Lips et al. 2005); hence, it can be assumed that nicotine can influence placental vascularisation and perfusion as well as nutrient transfer.

Cotinine passes just as readily into the fetal circulation and can accumulate there at nearly twofold concentrations, as placental perfusion studies have shown (Sastry 1991).

Cadmium acts as a metalloestrogen and endocrine disruptor in reproductive organs and during fetal development (Kawai et al. 2002). It accumulates in the placenta, resulting in twice as high tissue levels in smokers (Stasenko et al. 2010). It impairs placental hormone synthesis of both steroid and proteohormones.

Benzopyrenes are polycyclic aromatic hydrocarbons which, in high concentrations in cigarette smoke, can exert mutagenic effects in both active and passive smoking (Lee et al. 2011). They also pass via the placenta into the fetal blood and apparently modify placental metabolism (Sanyal and Li 2007).

Carbon monoxide (CO) is found in elevated blood concentrations in smokers, readily crosses the placental barrier and, like nicotine, can reach high fetal levels in the fetal compartment. It can ultimately cause placental and fetal hypoxia through the formation of carboxyhemoglobin (Rogers 2009).

The effects of tobacco consumption on the pregnant woman, placental morphology and function, and the fetus are shown schematically in ◘ Fig. 7.1.

7.2.2 Effects on Placental Morphology

Regardless of the type of tobacco consumption, smokers have smaller placentas with thicker villous membranes and increased trophoblast volume. The outer syncytiotrophoblast shows degenerative changes with increased apoptosis, necrosis and syncytial knots. The intervillous space is relatively reduced and at the same time, the villous capillaries are shortened with a smaller exchange surface (Ashfaq et al. 2008). These micromorphological changes reduce the transport capacity for nutrients and gas exchange.

Placental effects can be detected directly by Doppler sonography of maternofetoplacental perfusion. Dose-dependently, uteroplacental perfusion is worsened, umbilical artery resistance is increased and ultimately birth weight is decreased (de Machado et al. 2011) (◘ Fig. 7.2).

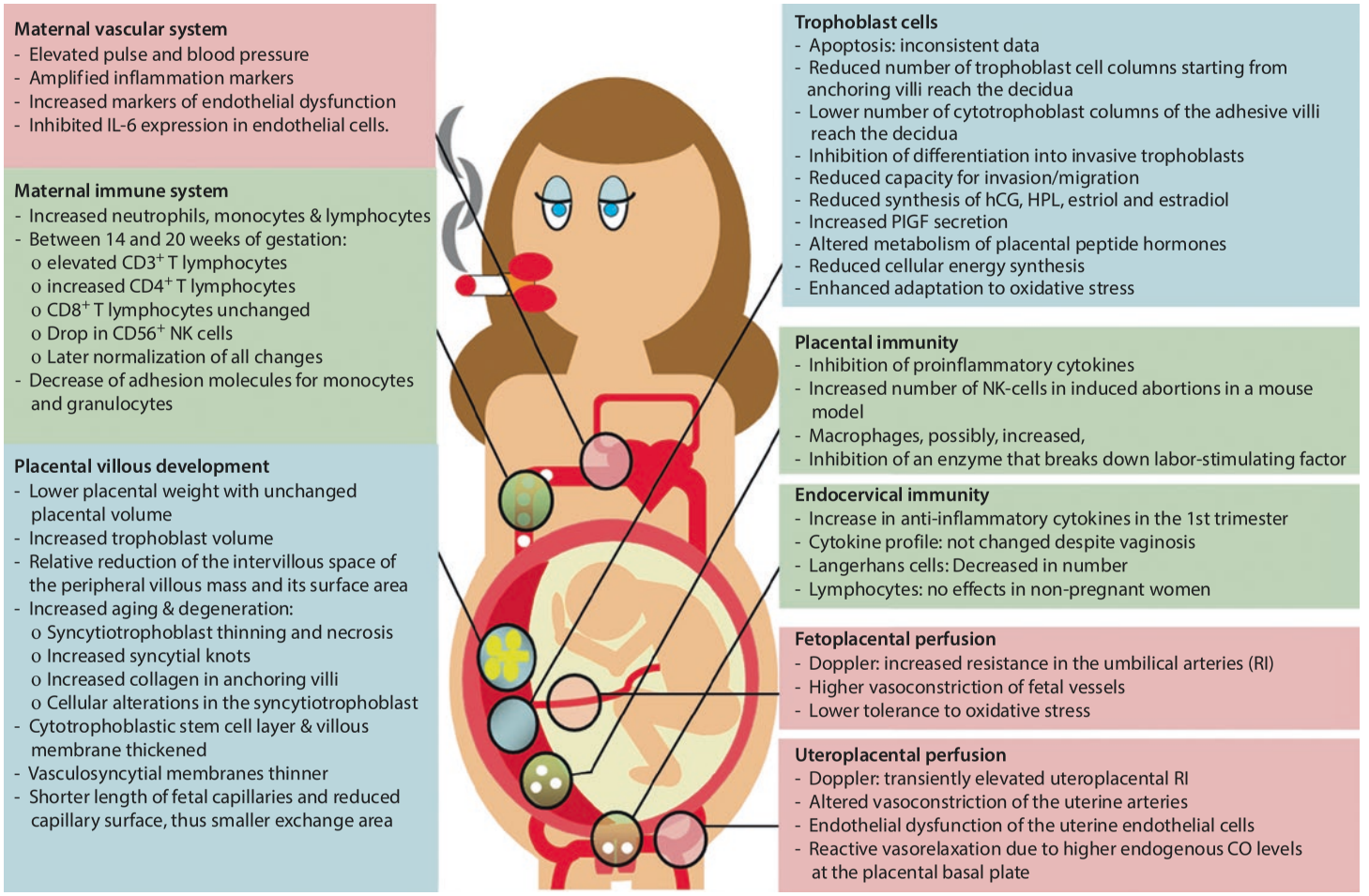

Fig. 7.1 Possible effects of smoking during pregnancy on placenta-associated compartments

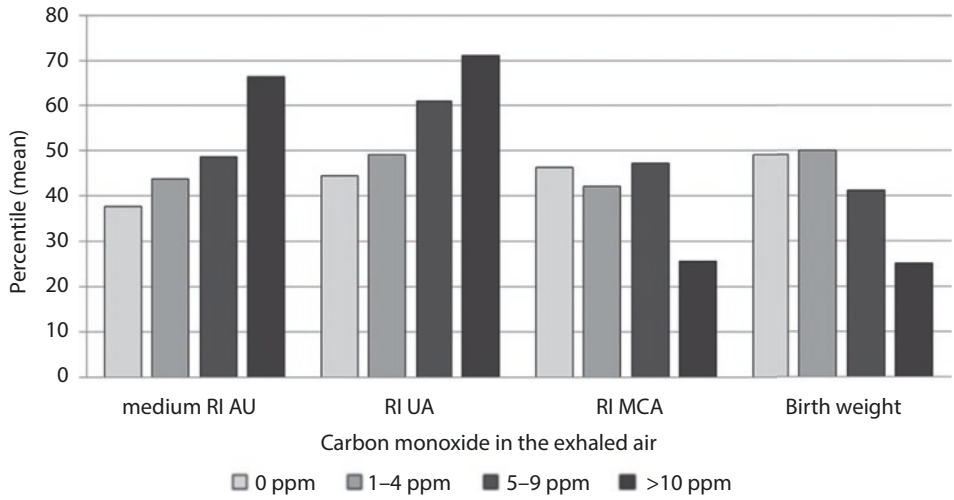

Fig. 7.2 Dependence of the placental perfusion resistance (resistance index, RI) on the maternal side (uterine artery, AU), the fetal side (umbilical artery, UA), the fetal cerebrovascular resistance (middle cerebral artery, MCA) as well as birth weight on the carbon monoxide concentration of exhaled air as a measure of smoking. The CO content of exhaled air is measured in parts per million ("ppm"). (Modified after de Machado et al. 2011) (Graphical representation of numbers, column chart)

7.2.3 Effects on Trophoblast Cells

Smoking may have different effects on the apoptosis rate. While increased trophoblast apoptosis was demonstrated in smokers, lower apoptosis was found in the syncytiotrophoblast. Carbon monoxide, on the other hand, has anti-apoptotic and anti-necrotic effects, at least in an in vitro model (Bainbridge et al. 2005).

Smoking appears to affect the balance between proliferation and differentiation of trophoblasts and particularly impairs the differentiation of invasive cytotrophoblasts (Jauniaux and Burton 2007). Cadmium decreases trophoblast proliferation, and benzopyrenes negatively affect trophoblast stem cell transcription (Xie et al. 2010).

However, not only differentiation into an invasive phenotype, but also trophoblast invasion directly is inhibited. Instead of invading the decidua starting from anchoring villi, a larger proportion of anchoring villi fail to reach the uterus and degenerate in the intervillous space (Genbacev et al. 2000). Nicotine inhibits I-selectin-mediated adhesion as well as the expression of fibronectin and its receptor factors, which are required for undisturbed migration and invasion of extravillous trophoblasts.

Placental hormone production of hCG (human chorionic gonadotropin), hPL (human placental lactogen), estriol and estradiol is lower in smokers, while the angiogenesis factor PlGF ("placental growth factor") is found increased in maternal serum (Zhang et al. 2011).

7.2.4 Oxidative Stress and Endothelial Dysfunction

The various tobacco components cause oxidative stress in the placenta. Maternal cotinine levels correlate with markers of maternal oxidative stress as well as with markers of placental DNA oxidation. The generation of free radicals simultaneously activates the antioxidant system.

In vivo studies demonstrate increased expression of heme oxygenase enzymes (HO-1 and HO-2) in the placental basal

plate of smokers, while in vitro HO-1 expression has been shown to be dose-dependent in trophoblasts (Sidle et al. 2007). HO-1 shows anti-inflammatory properties in addition to its antioxidant properties (Tranquilli and Landi 2010). HO expression is also increased several times in the myometrium of pregnant women, where it reduces both spontaneous and oxytocin-induced contractility (Acevedo and Ahmed 1998).

Nicotine reduced sFlt-1, s-endoglin and PlGF release in trophoblast cultures, whereas this was increased in HUVEC (Human Umbilical Vein Endothelial Cells) cultures (Romani et al. 2011). CO decreased sFlt-1 and s-endoglin production in endothelial cells and placental villous explants from preeclamptic pregnant women (Cudmore et al. 2007).

Furthermore, the activity of endothelial nitric oxide synthase (eNOS) in placental villous tissue and the umbilical endothelium is reduced in pregnant women who smoke, which may lead to a disturbance of NO-dependent vascular dilatation and thus directly to fetal insufficiency (Myatt et al. 1997).

In the pathogenesis of preeclampsia, an angiogenic imbalance between growth factors such as VEGF ("vascular endothelial growth factor") and PlGF and their vascular wall receptors (Flt-1) or soluble receptors (sFlt-1, s-endoglin) plays a central role. The concentration of the latter is substantially regulated by placental HO-1, as shown above. In addition, a higher concentration of PlGF has been demonstrated in female smokers. Via these mechanisms, the sFlt-1/PlGF ratio is reduced. This might explain why smokers selectively have a lower incidence of that form of preeclampsia, which is not associated with placental insufficiency and intrauterine growth restriction. However, the percentage of cases of early preeclampsia (<32 SSW) in heavy smokers is higher than in nonsmokers (28.5% vs. 20.1%), and 87% of these conditions are associated with FGR; this proportion is much lower in nonsmokers (47%).

7.2.5 Placental Transcriptome

Comparative analyses of placental expression patterns of smokers and nonsmokers reveal over 300 differentially expressed genes. In smokers, xenobiotic genes such as CYP1B1, GSTM1 and CBR3, which are also involved in the detoxification of carcinogens and environmental toxins, were upregulated. In contrast, genes involved in trophoblast invasion, proliferation and apoptosis but also cell adhesion and wound healing were deregulated in the placentas of smokers (Votavova et al. 2011).

7.3 Alcohol During Pregnancy

The prevalence of alcohol consumption among women of reproductive age is high in Germany, as it is worldwide. However, most women stop drinking alcohol after they become aware of their pregnancy. On the one hand, however, this may only be after critical weeks of early pregnancy, and on the other hand, there is a largely unknown high rate of pregnant women who continue to consume alcoholic beverages at least occasionally.

For Europe, a rate of 14–30% of pregnant women must be assumed who repeatedly consume alcohol knowingly. The most recent German data come from the GEDA study of 2012, in which 20% reported moderate and 8% risky alcohol consumption during pregnancy (AWMF 2016). As these data are based on interviews with mothers, a significant under-reporting can be assumed

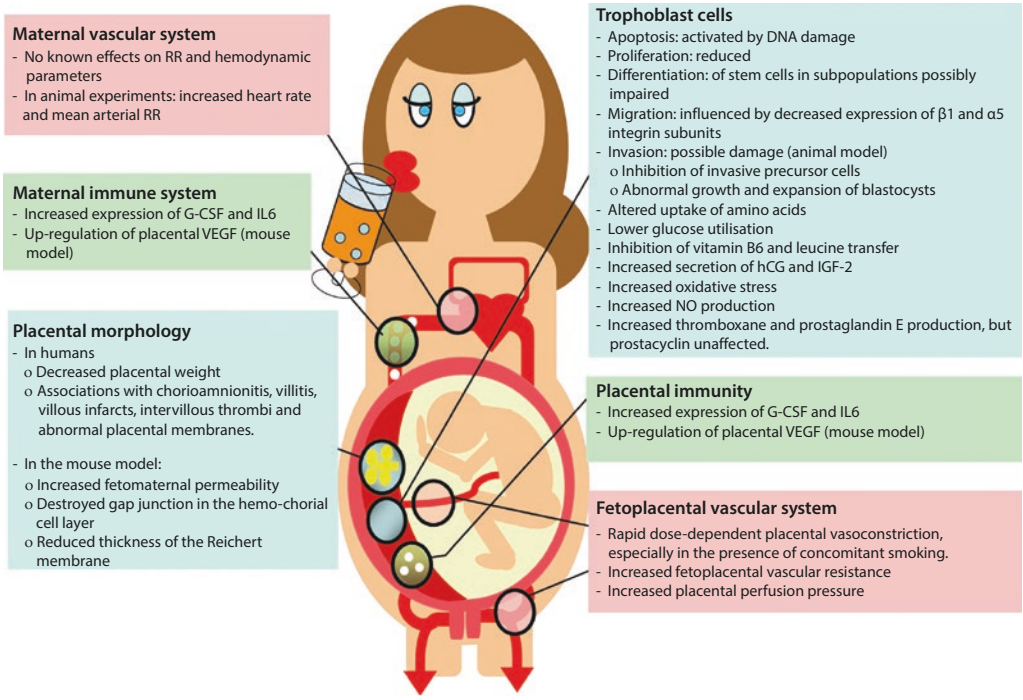

Fig. 7.3 Possible alcohol effects in pregnancy on placenta-associated compartments

due to the considerable societal pressure of expectations.

Although aldehyde dehydrogenase activity can also be detected in the placenta, ethanol is metabolized only to a very small extent (Burd et al. 2007). Since alcohol passes the placental barrier unhindered, the occurrence of the fetal alcohol spectrum disorder (FASD) must be expected in up to 2% of all children and the full-blown fetal alcohol syndrome (FAS) in approximately 0.2%. Detailed information on this most common congenital childhood disorder can be found in the S3 guideline "Fetal Alcohol Spectrum Disorder" (AWMF 2016).

The effects of alcohol consumption on the pregnant woman, placental morphology and function, and the fetus are shown schematically in Fig. 7.3.

7.3.1 Effects on Placental Morphology

Most studies show decreased placental weight and lower placenta/birth weight ratios in association with alcohol (Carter et al. 2016). Villitis, maternal chorioamnionitis, villous infarcts, and intervillous thrombi are also found with alcohol exposure (Baldwin et al. 1982). The placental barrier of the mouse becomes more permeable under acute alcohol exposure due to destroyed gap junctions and reduced thickness of the Reichert's membrane (Haghighi Poodeh et al. 2012).

In the placenta, alcohol causes rapid dose-dependent vasoconstriction, resulting in an increase in fetoplacental resistance (Burd et al. 2007).

7.3.2 Effects on Trophoblast Cells

Repeated alcohol exposure has a cytotoxic and apoptosis-inducing effect as a result of DNA damage in trophoblast cultures (Joya et al. 2015). Ethanol-induced apoptosis in human cytotrophoblasts is calcium channel dependent.

Ethanol appears to inhibit the proliferation of immature trophoblasts and also the differentiation of trophoblastic stem cells into different subpopulations (Kalisch-Smith et al. 2016).

At least in animal models, alcohol affects placentation and fetal growth in a dose-dependent manner by inhibiting invasive trophoblastic precursor cells, their cell adhesion and motility, and ultimately the transformation of maternal spiral arteries (Gundogan et al. 2015). Alcohol alters first-trimester trophoblast migration in vitro and associated beta-1 and alpha-5 integrin subunit expression (Rout 2006).

Alcohol inhibits the uptake and transport of taurine and other amino acids in vitro. In contrast, glucose uptake by trophoblast cells remains unaffected by alcohol, but not their glucose metabolism (Burd et al. 2007). In placental perfusion studies, alcohol had little effect on the maternofetal transfer of bioptin, histidine and thiamine, while vitamin B6 and leucine transfer were inhibited.

Chronic ethanol treatment decreased hCG and IGF2 secretion in a dose-dependent manner in trophoblast cell lines by increasing cAMP production (Joya et al. 2015). Interestingly, the same effect was found in human placental tissue after prenatal alcohol consumption.

7.3.3 Oxidative Stress and Endothelial Dysfunction

Markers for oxidative stress are present in trophoblasts and stromal cells of placental villi even after short-term exposure to alcohol. In particular, the NO signaling pathway and reactive oxygen species were involved and stress in the endoplasmic reticulum and DNA damage in the stroma were detectable (Repo et al. 2014).

In a placental perfusion study, alcohol exposure caused increased thromboxane production in both fetal and maternal circulation, while prostacyclin was unaffected (Burd et al. 2007).

In first trimester trophoblast cultures, alcohol led to increased cytokine expression (G-CSF, IL6) (Svinarich et al. 1998). In the mouse placenta, alcohol upregulates VEGF synthesis (Haghighi Poodeh et al. 2012).

7.4 Methamphetamines and MDMA

The abuse of designer drugs such as methamphetamine (METH, crystal) and 3,4-methylenedioxy-N-methamphetamine (MDMA, ecstasy) has also increased extremely in Germany in the last decade, so that these drugs now cause the greatest problems in pregnant women after alcohol and nicotine (Fig. 7.4) (Dinger et al. 2017).

Almost 85% of the pregnant women with methamphetamine or opioid abuse are under 30 years of age at the time of delivery and thus significantly younger than the German average, but do not differ from other mothers in terms of their socioeconomic and educational status. The fact that prenatal care was perceived to a much lesser and irregular extent has to be seen particularly critically under the knowledge of the high perinatal risks.

METH abuse is associated with a higher risk of preterm birth (OR 4.11; 95% CI 3.05–5.55), lower birth weight (OR 3.97; 95% CI 2.45–6.43) and fetal growth restriction (OR 5.79; 95% CI 1.39–24.06) (Ladhani et al. 2011). In the Saxon cohort, the pre-

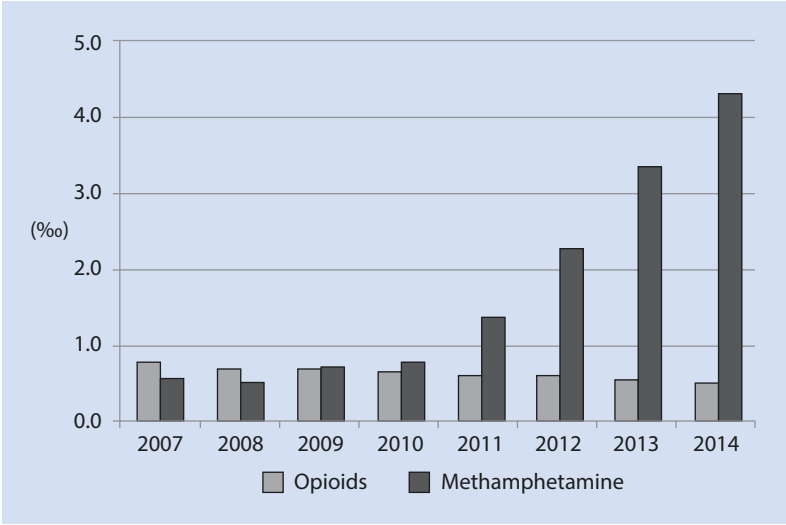

Fig. 7.4 Proportion of inpatient newborns in Saxony in whom methamphetamines or opioids were detected. (After Dinger et al. 2017)

term birth rate of 32% was also 4-fold higher. Microcephaly had to be registered in 22%, malformations of the CNS in 26% and cardiac anomalies in 12% of the newborns (Dinger et al. 2017). Intrauterine fetal death and placental abruption occurred significantly more frequently.

METH is metabolized in the maternal liver to amphetamine and 4-hydroxymethamphetamine, and both readily cross the placental barrier (Ross et al. 2015). Due to the methyl group, METH is more lipophilic and thus can cross biological membranes more quickly. METH has a higher dependence potential than other psychostimulants because it triggers the serotoninergic mental "reward mechanism" and floods the brain with dopamine. In doing so, both its reuptake from the synaptic cleft and its intracellular degradation by monoxidases are inhibited. MDMA also acts as a hallucinogen by not only increasing intracellular serotonin, dopamine and noradrenaline concentrations, but also by binding directly to their receptors.

METH and MDMA cross the placental barrier unimpeded and are actively transported by the serotonin and noradrenaline transporters in the syncytiotrophoblast. Due to competitive displacement, this simultaneously leads to the increase of neurotransmitters in the intervillous space, resulting in vasoconstriction, uterine contractions and platelet activation. On instrumented sheep, methamphetamine passed the placental barrier as early as 30 sec after i.v. administration and accumulated at higher fetal concentrations than in maternal blood because of its longer half-life (Burchfield et al. 1991). The tissue/serum ratio after 2 h was highest in the fetal lungs, followed by the placenta. Methamphetamine caused a fetal blood pressure increase of up to 37% with concomitant decreases in arterial pH and fetal O_2 saturation. The impaired transplacental O_2 transfer is due to a dose-dependent decrease in uteroplacental perfusion based on an increase in both uterine and umbilical vascular resistance (Stek et al. 1995).

The frequency of pregnancy complications in methamphetamine abuse suggests impaired placental function, although studies of this are rare. Unlike other drugs, methamphetamine-dependent pregnant

women have been found to have higher placental weights and, with concomitant fetal growth restriction, a higher placenta/birth weight ratio, possibly as a compensatory mechanism for the worsened fetal supply due to vasoconstriction (Carter et al. 2016). Thus, growth restriction is also severe when fetal intoxication is still detectable at birth compared to abusiveness in the first trimester only (Forray and Foster 2015).

7.5 Cocaine

The 2013 UN Office on Drugs Crime Report describes North America, western and central Europe as the main cocaine markets. Although cocaine use has decreased, it remains the second most important illicit drug in Europe, with an estimated 1.2% prevalence.

Due to its lipophilic nature, cocaine passively crosses the fetomaternal barrier and the fetal blood-brain barrier unimpeded. The most common pregnancy complications of cocaine exposure are preterm birth, premature rupture of membranes, preeclampsia, fetal growth restriction and placental abruption (Ross et al. 2015). A specific fetal malformation pattern is not discernible, but children of cocaine-dependent mothers show a smaller head circumference and more frequently display behavioral disorders already in infancy, which together with cognitive impairments, in particular of language development, persist into adolescence.

The placenta-associated pregnancy complications can be explained by various cocaine effects. For example, cocaine directly blocks norepinephrine reuptake in synaptic neurons and myometrium, resulting in increased dopamine, serotonin, and norepinephrine concentrations in the synaptic cleft. The resulting vasoconstriction increases maternal blood pressure with concomitant worsened uteroplacental perfusion. Similarly, direct effects in the placenta are known. As in the autonomic nervous system, cocaine affects noradrenlin and serotonin transporters in the syncytiotrophoblast, so that in turn higher monoamine levels in the intervillous space worsen its perfusion and increase uterine contractility (Ganapathy 2011). In addition, active amino acid transport across the placenta as well as hCG synthesis are impaired (Malek et al. 2009). However, cocaine also increases the shedding of syncytiotrophoblast microparticles into the maternal circulation, which play a central role in the pathophysiology of preeclampsia.

Ultimately, placental progesterone synthesis is also inhibited after cocaine exposure, mainly through inhibition of the P450scc-dependent enzyme cascade of progesterone synthesis from cholesterol. At the same time, cocaine blocks the sigma receptor in the endoplasmic reticulum of placental cells, for which progesterone is a natural ligand (Ganapathy 2011).

7.6 Opiates

The rate of opiate addiction seems to be relatively constant in Europe and Germany. In Germany, about one in three people treated for addiction is addicted to heroin or opiates, with women more often affected than men (39% vs. 34%). Of the clients with primary opioid problems, about one in four also had an alcohol disorder or cocaine abuse (22.4%) in 2014 (German Ministry of Health [Bundesministerium für Gesundheit] 2016). In addition, opioids are also used medically for pain and substitution therapy in about 1% of all pregnant women (Engeland et al. 2008).

7.6.1 Placental Transfer

Since endogenous and synthetic opioids have different structures, placental permeability depends on their lipid and water solubility. Polar opioids pass slowly across the placental barrier into the extracellular fluid space, accumulate in the amniotic fluid and subsequently in the fetal intestinal tract. Lipophilic opioids, like most substances used as drugs, are rapidly transported across the placenta via transcellular pathways and placentofetal distribution depends mainly on their protein binding (Malek and Mattison 2011).

Morphine is rapidly exchanged across the placenta and reaches equilibrium between mother and fetus within 5 min, so that its cord blood concentration equals that in the maternal blood. The same is true for the synthetic opioids fentanyl and sufentanyl, which reach their maximum concentration in fetal blood after 5 min of maternal bolus administration, but can also be detected in the placental intervillous space. The transfer of methadone and its clearance index are greater in the fetomaternal direction than in the maternofetal direction, with a substantial fraction remaining in placental tissue. This may be explained by the unidirectional activity of the efflux transporter P-glycoprotein (P-GP), which is found in high expression in trophoblast tissue. Placental perfusion experiments after premature birth showed a significantly lower methadone transfer rate with simultaneously increased P-GP expression compared to mature placentas.

7.6.2 Effects on Trophoblast Cells

Opiate dependence is known to have a higher incidence of all placenta-associated pregnancy complications (preeclampsia, PROM, placental insufficiency, placental abruption, FGR and IUFD) (Ross et al. 2015). This also applies to the long-term use of opiates for therapeutic reasons.

Opiate receptors are G-protein coupled receptors and can be divided into three groups: mu (μ), delta (δ) and kappa (κ). κ- and μ-receptors are found in human placenta, and respective direct effects of opioids on trophoblast cells have been demonstrated in vitro (Yazdy et al. 2015). While κ-opioid receptor stimulation increases the release of hPL and hCG from trophoblast cells, stimulation of μ-receptors, e.g. by morphine, induces NO production. However, activation of κ-opioid receptors also inhibits acetylcholine release, which is a mediator of placental amino acid transfer and placental perfusion important for fetoplacental supply. Thus, FGR in opiate abuse may be due to both impaired amino acid transfer across placental villi and decreased uptake on the fetal side of the placenta. Because heroin increases shedding of syncytiotrophoblastic microparticles into the maternal circulation, this could explain the clustering of preeclampsia in heroin-dependent pregnant women (Malek and Mattison 2011).

Placental aromatase, as a cytochrome P450-dependent enzyme, plays a central role in the metabolism of opioids, but it is also the key enzyme in the biosynthesis of estrogen in the placenta. Thus, some opioids are competitive inhibitors of the aromatization of dehydroepiandrosterone (DHEAS) and testosterone into estradiol and estriol.

7.7 Cannabis

According to data from the drug affinity studies of the Federal Centre for Health Education, 10.2% of adolescents and 34.7% of young adults aged 18-25 in Germany have used illicit drugs at least once in their lives. The incidence of abuse or dependence in the adult population is 1% with regard to cannabis and well below 1% with regard to other illicit drugs. Moreover, the use of cannabis

accounts for the predominant share of illicit drug use in Germany. The lifetime prevalence among young women for at least one-time cannabis use is 8.2% among adolescents and 26.6% among young adults (German Ministry of Health [Bundesministerium für Gesundheit] 2016). In the USA, about 5% of pregnant women smoke marijuana during the first trimester and another 1.5% during the third trimester (Ross et al. 2015).

7.7.1 Placental Transfer

Cannabis (marijuana, hashish) is derived from the hemp plant Cannabis sativa and is usually smoked. Cannabinoids are a group of over 80 chemically similar but differently acting constituents of the hemp plant, the most important being THC (tetrahydrocannabinol) and CBD (cannabinidiol). While THC has strong psychoactive, euphoric, analgesic and antiemetic effects, as well as altering perception, CBD has hardly any psychoactive effects, but is antianxiety, antiemetic and anticonvulsant, so that it is also used medically. THC binds to the G-protein-coupled cannabinoid-1 (CB-1) and cannabinoid-2 (CB-2) receptors, which are detected in the endometrium, myometrium, and also placental tissue. Their activation can cause disturbances in placentation and pregnancy outcomes (Brents 2016). THC is lipophilic and is stored in adipose tissue, so its elimination can take up to 30 days and THC can also be found in breast milk. THC inhibits the placental capacity of drug elimination, e.g. of other drugs that are frequently coconsumed.

Cannabis use in early pregnancy is not teratogenic. There are also no increased premature births or neonatal adjustment disorders. Unlike other drugs, there is no typical neonatal withdrawal syndrome and no increased neonatal mortality (Gunn et al. 2016). However, there is a clear association with low birth weight and FGR, although co-factors such as tobacco, other drugs, low educational and socioeconomic status must be taken into account (Carter et al. 2016).

Nevertheless, both in animal experiments and in humans, larger placental weights are found with cannabis exposure (Carter et al. 2016). Macroscopically, there are no differences in the mature placenta, but microscopically, the diameters of the umbilical vessels are wider than in non-users (Costa 2016). In contrast, THC causes vasoconstrictive effects in the placental microcirculation as well as decreased fetal oxygenation. Chronic THC abuse affects placental absorption of nutrients as well as of folic acid. Acute exposure, on the other hand, increases folic acid uptake.

7.7.2 Effects on Trophoblast Cells

THC also has a direct cellular effect in syncytium and cytotrophoblast cultures. At low doses, it increases metabolic activity, while at high doses it decreases cell viability and disrupts trophoblast syncytialization (Costa 2016). Activation of CB receptors inhibits trophoblast cell syncytialization in vitro by decreasing the expression of the signaling molecules syncytin-1 and -2 (Costa 2016).

At THC concentrations comparable to those of occasional users, proliferation of BeWo cell cultures is inhibited and transcriptional processes encoding growth, apoptosis, cell morphology, and ion exchange channels are activated. However, these gene expression patterns differ from those in placentas of hypoxia-associated FGR and preeclampsia, so these THC effects are likely to be effected via other pathways.

Some of these effects do not appear to be CB receptor-mediated. The more cytoprotective effects of low doses and cell death at high doses may be attributed to influences of oxidative/nitrate-dependent stress mechanisms and mitochondrial metabolism (Costa 2016).

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Placenta-Related Hemorrhage: Pathophysiology, Diagnostics, Management

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8.1 The Placenta Accreta Spectrum (PAS)

Thorsten Braun and Wolfgang Henrich

8.1.1 Introduction

As a potentially life-threatening complication, the placenta accreta spectrum (PAS) contributes significantly to perinatal morbidity and mortality. The incidence of PAS, currently one in 533 to one in 2510, is increasing in parallel with the increase in the rate of cesarean deliveries (ACOG 2015). The highest risk is described for women with placenta praevia and previous cesarean sections. Histopathologically, placenta accreta is distinguished from placenta increta and percreta. Clinically relevant, placenta accreta can be distinguished from placenta increta/percreta. In placenta accreta, complete removal of the placenta is possible after manual detachment or curettage, while in placenta increta/percreta, this is only possible by additional surgical intervention and is associated with increased maternal morbidity. Maternal and neonatal morbidity and mortality can be reduced in particular by prenatal ultrasound diagnosis as a screening procedure and subsequent interdisciplinary planning of delivery in a tertiary care center. Magnetic resonance imaging (MRI) can be used to substantiate and/or confirm the findings. In 2012, due to the lack of prospective randomized studies on the diagnosis and treatment of PAS, 13 European centers with 42 specialized obstetricians, gynecologists, pathologists, and basic researchers joined forces (European Working Group on Abnormally Invasive Placenta, EW-AIP) and in 2017 the International Society for Abnormally Invasive Placenta (IS-AIP, ▶ www.is-aip.org) was founded. The aim of this group is the prospective and centralized registration of all PAS cases in Europe to optimize the knowledge regarding the etiology, diagnosis and therapy of PAS.

8.1.2 Epidemiology

Until the 1950s, PAS was a rare event, occurring in one in 30,000 births (Read et al. 1980; Miller et al. 1997). Then, beginning in the 1980s and 1990s, there was a successive up to 10-fold increase in incidence to one in 731 deliveries in 2008 and 2011 in a cohort of 115,000 births (Miller et al. 1997; Wu et al. 2005). The incidence was even higher at one in 695 deliveries in Canada in 2009/2010 (Khong 2008) and is currently about three in 1000 births (Belfort 2010). According to a projection, a total of 4504 PAS cases must be expected in the USA in 2020, which could be associated with 130 additional maternal deaths (Solheim et al. 2011).

8.1.3 Risk Factors

The main risk factor cited is the increasing rate of cesarean sections (C-sections) (Oyelese and Smulian 2006a, b). Uterine surgeries such as repeated curettages, myoma enucleation, resection of septa or synechiae, a history of endometritis, or former placental abruption, isthmic resection after interstitial extrauterine pregnancy in the tubal angle, cesarean scar pregnancy, maternal age >35 years, pelvic irradiation, reproductive interventions such as in vitro fertilization, and female gender of the fetus are associated with an increased risk of developing PAS (Henrich et al. 2008; Doumouchtsis and Arulkumaran 2010; Esh-Broder et al. 2011; Fitzpatrick et al. 2012; Hayashi et al. 2012; Nageotte 2014; Timor-Tritsch et al. 2014; Kaser et al. 2015; Silver et al. 2015). The most common constellation of PAS is placenta praevia following cesarean delivery (Wu et al. 2005) (◘ Table 8.1).

Table 8.1 Risk factors and associated incidence of the placenta accreta spectrum (PAS). (Clark et al. 1985; Miller et al. 1997; Silver et al. 2006a, b; NIH 2010)

Risk factor	Risk of PAS (%)
One previous C-section	0.3
Two previous C-sections	0.6
Three previous C-sections	2.4
Placenta praevia and	
– no previous C-section	1–5
– one previous C-section	11–25
– two previous C-sections	35–47
– three previous C-sections	40
– four or more previous C-sections	50–67

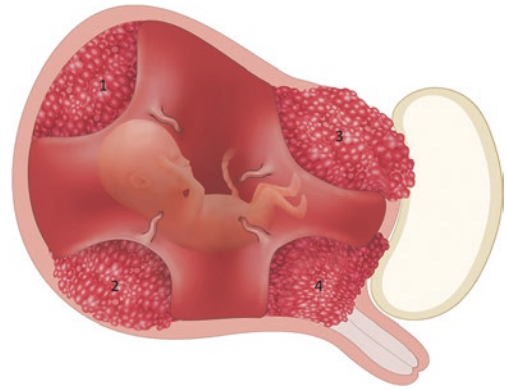


Fig. 8.1 Schematic representation of different degrees of severity (depth of invasion, localization and vascular extent) of the placenta accreta spectrum. (*1*) Placenta accreta with invasion of the trophoblast beyond the decidua basalis to the border of the myometrium; (*2*) Placenta increta with invasion of the trophoblast deep into the myometrium; (*3*) Placenta percreta with invasion of the trophoblast into the myometrium to the border of the serosa or beyond with or without infiltration of adjacent structures; (*4*) Placenta increta/percreta with cervical invasion and bladder wall involvement

8

8.1.4 Definition

In defining the implantation disorder of the placenta, a distinction is made between the histopathological definition obtained postpartum and the prepartum and peripartum clinical definition. Histopathologically, a differentiation can be made between placenta accreta, increta and percreta depending on the extent and depth of invasion of the chorion frondosum into the myometrium (Fig. 8.1). **Placenta accreta** shows invasion of the trophoblast to the myometrial inner wall with disruption or absence of the decidua basalis. **Placenta increta** shows invasion of the trophoblast deep into the myometrium but not beyond the serosa, and **placenta percreta** shows invasion up to the uterine serosa or beyond the uterine borders and the serosa with or without infiltration into neighboring organs such as the urinary bladder, the parametria, or the intestine. In an analysis of a total of 138 histologically confirmed PAS cases, the frequency distribution was as follows: placenta accreta 79%, placenta increta 14% and placenta percreta 7% (Miller et al. 1997; Wu et al. 2005).

According to the clinical relevance, however, only two groups are distinguished, placenta accreta and placenta increta/percreta. In the case of placenta accreta, which requires a manual detachment or curettage, the placenta can be removed completely and no further surgical interventions are necessary. In the case of placenta increta/percreta, the placenta cannot be completely removed by manual detachment or post curettage. Further surgical interventions are required (Henrich and Braun 2013), and these are associated with increased maternal mortality of up to 7% overall (O'Brien et al. 1996).

This must be distinguished from a placenta which, in the area of scar dehiscence, for example in the area of the old C-section scar, bulges out onto the surface of the uterus and is only covered by the uterine serosa (Fig. 8.2). This is not a classical PAS and its treatment is usually much less complicated.

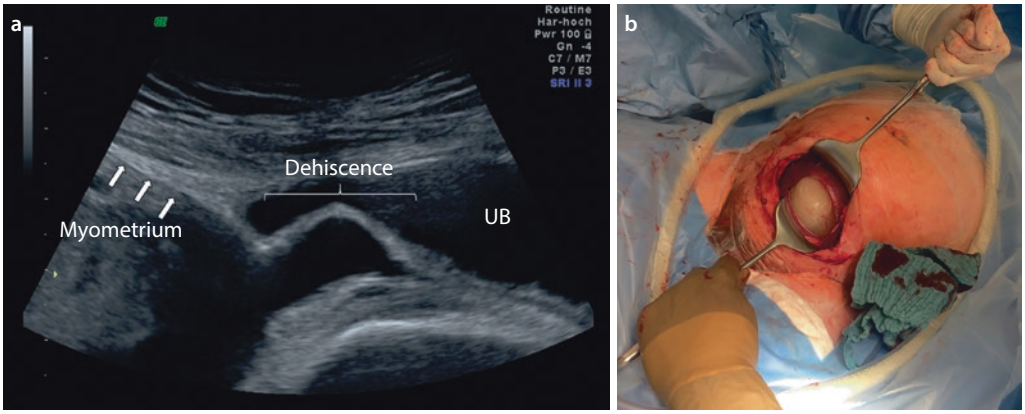


Fig. 8.2 **a, b** Uterine scar dehiscence. **a** Transabdominal sonography. **b** Intraoperative findings. *UB* Urinary bladder

In the English literature, all degrees of severity are often summarized under the generic term "placenta accreta". This complicates the comparability of studies with clearly different clinical significance.

8.1.5 Pathogenesis

The exact pathogenesis of PAS is not yet known. One theory describes a very thin, interrupted or missing decidua basalis as the cause (Khong 2008) and attributes this to previous intrauterine interventions (Tantbirojn et al. 2008). After all, one study showed an association with previous intrauterine interventions such as cesarean section, curettage and/or myoma enucleation in 80% of PAS cases (Tantbirojn et al. 2008). Other theories assume an overshooting, non-regulated extravillous trophoblast invasion or a disturbed maternal vascular remodeling in the area of a uterine scar (Wehrum et al. 2011; Chantraine et al. 2012; Jauniaux and Jurkovic 2012). The duration of pregnancy seems to play a minor role in this regard for the depth of implantation, as there are already histopathologically confirmed findings of a placenta percreta from the 14th week of gestation (Pron et al. 2005; Hamar et al. 2006). Another theory for the development of PAS is based on the preferential nidation of the placenta in the area of hypoxemic tissue areas, which are found in particular in scar tissue after intrauterine surgery (Rosen 2008).

8.1.6 Diagnosis

The antepartum diagnosis of PAS remains a challenge for the investigator even today (Tutschek et al. 2014). In a large population-based study, PAS was not detected antepartum in a total of 50–70% of cases (Tikkanen et al. 2011; Thurn et al. 2016). However, it is the prenatal diagnosis of PAS and the interdisciplinary birth planning based on this diagnosis that allows the significantly improved surgical outcome and maternal survival. For example, antepartum diagnosis decreases maternal blood loss compared to pregnant women in whom PAS was only discovered perioperatively (Warshak et al. 2010; Tikkanen et al. 2011; Chantraine et al. 2013). The antepartum differentiation of the severity of PAS, in particular the differentiation of placenta accreta versus placenta increta with the

resulting clinical consequences, remains difficult despite increasing awareness of this issue. In addition to taking a detailed medical history and recording the known risk factors (Table 8.1), prenatal ultrasound and, in addition, MRI are used for diagnostic purposes.

Ultrasound diagnosis of PAS by means of B-scan and color or power Doppler sonography offers an irreplaceable imaging procedure to detect this life-threatening disease. In general, it is recommended to perform transabdominal and transvaginal sonography when the urinary bladder is moderately filled. The use of 12 MHZ transducers allows a high resolution especially in the near field. Already in early pregnancy between 5 and 7 weeks of gestation, a scar pregnancy should be ruled out and the position of the chorion in relation to the sectional scar should be assessed sonographically (Fig. 8.3) (Armbrust et al. 2015). A scar pregnancy in the first trimester with either an anterior myometrial thickness <2 mm (Kaelin Agten et al. 2017) or a location of the amniotic cavity relative to the midline axis of the uterus (Timor-Tritsch et al. 2016) are definitive predictors of the development of PAS and require early termination of pregnancy if a sectional scar pregnancy is confirmed (Jurkovic et al. 2016).

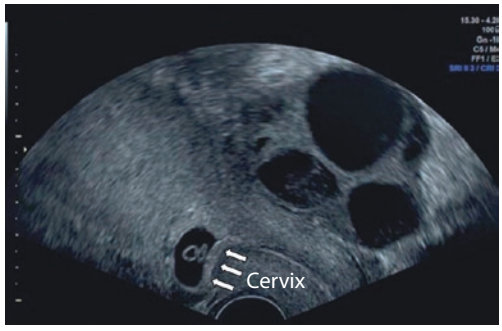


Fig. 8.3 Scar pregnancy. Transvaginal sonography showing an early pregnancy in the C-section scar region (*arrows*). (Modified after Armbrust et al. 2015)

Ultrasound for the Diagnosis of PAS

Numerous sonographic signs are mentioned as indicative of PAS, some from empirical studies and some supported by histological studies (Chantraine et al. 2012). However, the comparability of studies is limited due to different terminology of ultrasound signs (Jauniaux et al. 2016). In 2016, a unifying definition of ultrasound signs used in the literature was published by the EW-AIP (Table 8.2), without weighting their diagnostic value in detail (Collins et al. 2016). These are described below.

Thinning or Absence of the Myometrium

Sonographically, PAS shows a reduced or absent hypoechogenic myometrium above the placenta with ≤1–2 mm (Fig. 8.4). The visualization of the myometrium is highly dependent on the angle of insonation, the position of the fetal head and the compression this may cause of the uterine segment being examined, as well as by the gestational age at the time of the examination. In the literature, different thicknesses are given for a normal, preserved myometrial thickness (Comstock et al. 2004). One suggestion for standardizing the measurement of myometrial thickness is to measure in the region of the lower uterine segment between the bladder wall and the subplacental vessels, which can be visualized using color Doppler (Twickler et al. 2000; D'Antonio et al. 2014).

Placental Protrusion

Deviation of the uterine serosa from the expected contour, caused by abnormal protrusion of placental tissue into adjacent organs, typically above the fold of the bladder envelope or subvesically, should be interpreted as a sonographic sign of PAS. The uterine serosa appears intact, but the surface is bulged out (Fig. 8.5).

Table 8.2 Compilation of a unified definition by the European Working Group on Abnormally Invasive Placenta (EW-AIP) of the ultrasound signs of PAS currently used in the literature. (Modified according to Collins et al. 2016)

Ultrasound Sign	EW-AIP Standardized Proposals to Unify Definitions
2D B-scan	
Thinning or absence of the myometrium	Myometrial thinning ≤1–2 mm above the placenta or absence of any myometrial wall.
Placental protrusion	Deviation of the uterine wall due to protruding placenta into neighboring organs, typically in or above the bladder wall or parametrically. The uterine serosa appears intact, but the surface is distorted
Loss of the ”echo-poor zone”	Loss or irregular hypoechogenic zone between myometrium and placenta
Absence of the ”separation sign”	Absence of a “separation sign” between the uterine wall and the placenta, characterized by the absence of the hypoechogenic zone after dynamic compression and decompression by means of the transducer
Abnormal placental lacunae	Presence of numerous lacunae of different sizes (similar to moth damage) with irregular appearance, often with turbulent flow
Urinary bladder wall disruption	Disruption or loss of the bladder wall with absence of the hyperechogenic boundary layer between the myometrium and the bladder wall
Focal, expophytic excrescence	Placental tissue that breaks through the uterine serosa and can be visualized beyond it. Usually seen within a filled urinary bladder (very rare)
2D Color Doppler	
Vessels feeding the placental lacunae (“lacunae feeder vessels”)	Large caliber, blurred placental lacunae (similar to moth damage) with feeding vessels, blood flow partly with high velocity
Subplacental hypervascularity	Distinct color Doppler signal in the placental bed. This sign possibly indicates a high number of densely arranged, tortuous vessels in this region (evidence of multidirectional flow and aliasing effects)
Uterovesical hypervascularity	Distinct color Doppler signal between the myometrium and the posterior wall of the urinary bladder as a sign of vascular convolutions with multidirectional flow
Bridge vessels	Vessels that extend from the placenta beyond the myometrium and uterine serosa into the urinary bladder wall or other organs. These often run at right angles to the myometrial plane due to neovascularization on the uterine surface
3D Ultrasound[a]	
Intraplacental and uterovesical hypervascularity	Complex, irregular arrangement of numerous placental vessels, which are strongly tortuous and present with different vessel diameters
Bridge vessels	(As in 2D)
Placental protrusion	(As in 2D)
Focal, expophytic excrescence	(As in 2D)

[a]3D ultrasound ± angio or power Doppler mode, glass-body-mode (like 2D Doppler with the possibility of spatial imaging), VCI mode (contrast enhancement between boundary layers)

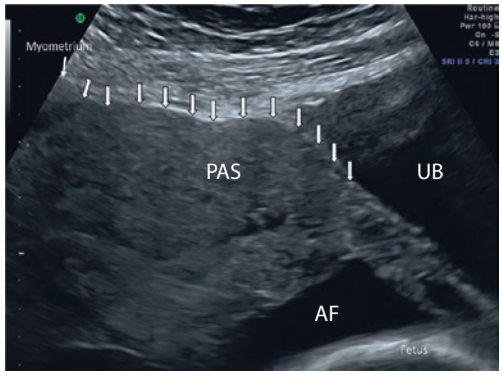


Fig. 8.4 Thinning or absence of the myometrium. Sonographically, PAS shows reduced or absent hypoechogenic myometrium above the placenta with ≤1–2 mm or less. *UB* Urinary bladder; *AF* Amniotic fluid

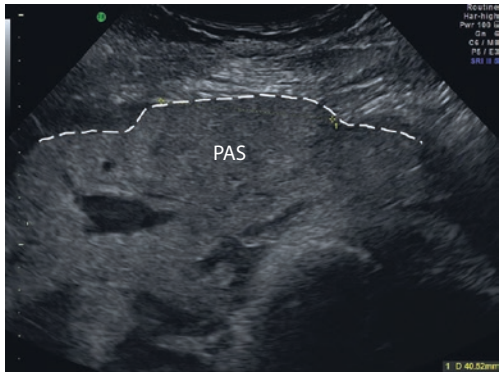


Fig. 8.5 Protrusion of the placenta. The uterine serosa deviates from the expected area caused by abnormal protrusion of placental tissue. Uterine serosa is intact but shows a raised contour

Focal Expophytic Excrescence

This sign is defined as ultrasound-visible placental tissue which breaks through the uterine serosa and can be visualized beyond it. Extremely rarely, placental tissue can be visualized within a filled bladder. This indicates bladder wall perforation with protrusion of placental tissue into the bladder lumen (D'Antonio and Bhide 2014). However, when the bladder is only marginally filled, the focally distended wall of a normal bladder may mimic such a space-occupying lesion. Therefore, the examination should always be done when the urinary bladder is moderately filled. Sonographic diagnosis of pure bladder wall invasion is even more difficult. In this regard, 3D ultrasound is a useful adjunct to assess the extent of bladder invasion (Chou et al. 2009). In 3D angiomode and glass-body mode, the complex arteriovenous vascular architecture can be visualized plastically.

Loss of the Clear Zone

The term "loss of the clear zone" describes the loss of an irregular, hypoechogenic separation layer in the area of the myometrium below the placental bed (Fig. 8.6). In 2014, Tutschek et al. coined a new sign, the Separation Sign, in this context (Fig. 8.7), which describes a dynamic phenomenon and is well suited for examining the placenta lying against the anterior wall (Tutschek et al. 2014). The myometrium with the underlying placenta are compressed by pressure with the transducer placed over the suspect site. This is possible transabdominally but also transvaginally. If the pressure is then reduced, normal separation of the placenta presents with visualization of the low echo zone between the placenta and the myometrial wall. This zone corresponds to the normal decidua and the subplacental myometrium with the respective subchorial

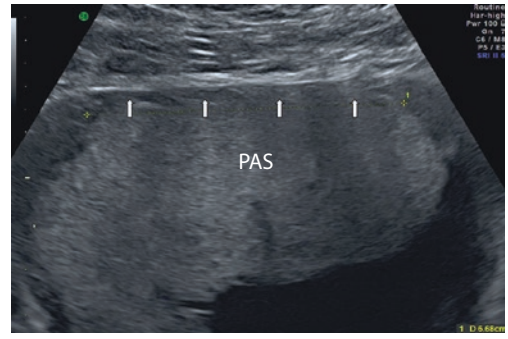


Fig. 8.6 "Loss of the clear zone". Loss of a hypoechogenic zone between the myometrium and the placenta

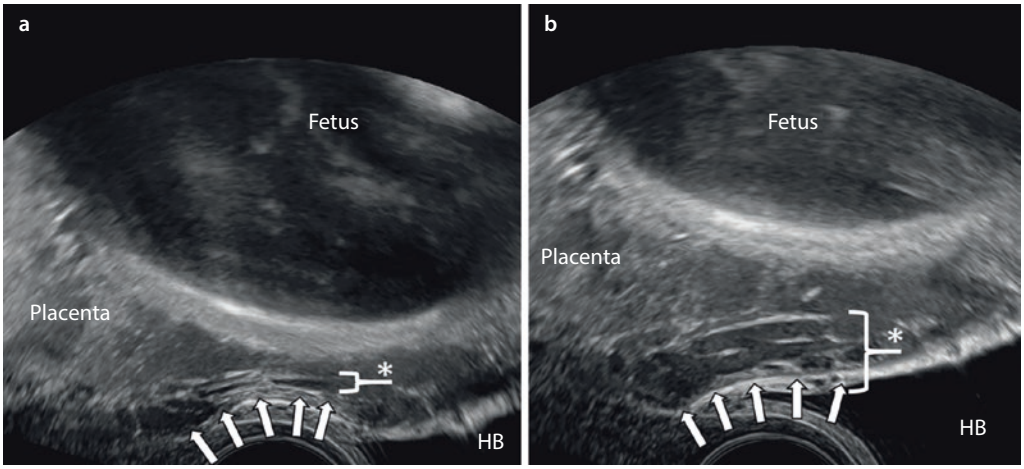


Fig. 8.7 **a, b** Separation Sign. The myometrium together with the underlying placenta is compressed by pressure with the transducer placed over the suspicious area **a**. If the pressure is then reduced, normal separation of the placenta is seen with visualization of the low echo zone (*) between the placenta and the myometrial wall **b**. This zone corresponds to the normal decidua and subplacental myometrium with their respective vessels. In the area of a PAS this separation cannot be visualized, there is a loss of normal tissue separation. *UB* Urinary bladder

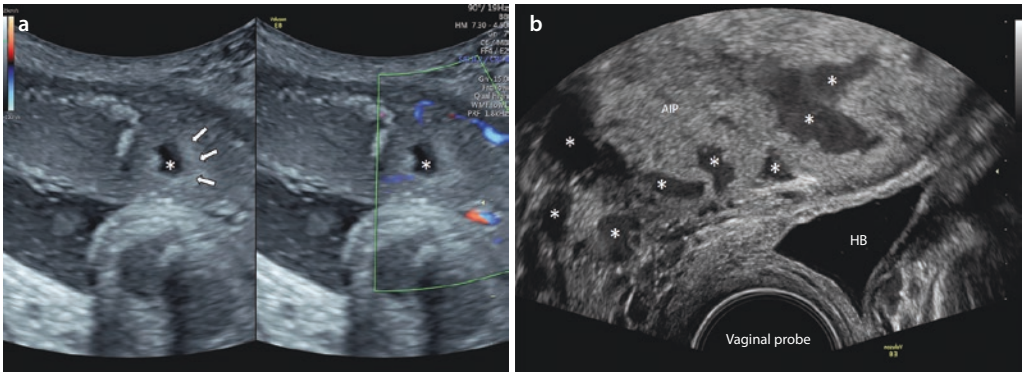


Fig. 8.8 **a** Normal lacunae with echogenic rim. **b** Abnormal placental lacunae looking as having moth damage in PAS. * Placental lacunae; *UB* urinary bladder; *arrow* echogenic rim

vessels. However, in the area of a PAS, this separation cannot be visualized, and there is a loss of normal tissue separation (Tutschek et al. 2014, with demonstration video online).

Abnormal Placental Lacunae

Lacunae in the placenta, which represent dilated venous spaces and presumably result from regressive changes and focal apoptosis, can be visualized in many normal placentas with high-resolution ultrasound equipment. Harmless lacunae are characterized by a hyperechogenic rim, internal echoes with partially thrombosed blood, and sluggish flow (Fig. 8.8a). Large and especially lacunae looking as having moth damage without a hyperechogenic border and with massive perfusion as well as contact to the uterine wall and possibly to the uterine surface represent an important feature of a PAS (Fig. 8.8b).

Urinary Bladder Disruption

Sonographically, the B-scan shows loss or disruption of the hyperechogenic ligament or "line" between the uterine serosa and the urinary bladder lumen (Fig. 8.9). Overall, transmural invasion into the urinary bladder is rare. Clinically, this can be discernible by macrohematuria. In particular, anterior placenta praevia with PAS may infiltrate or perforate the bladder wall as placenta percreta (Abbas et al. 2000; Oyelese and Smulian 2006a, b). In transvaginal examination of the anterior uterine wall and the urinary bladder wall with the vaginal ultrasound probe, similar to the separation sign described previously, the absence of displacement of these two layers can be observed in PAS with tangential pressure on the region and subsequent pressure reduction (Tutschek et al. 2014). With normal placentation, displacement of these layers can be readily observed.

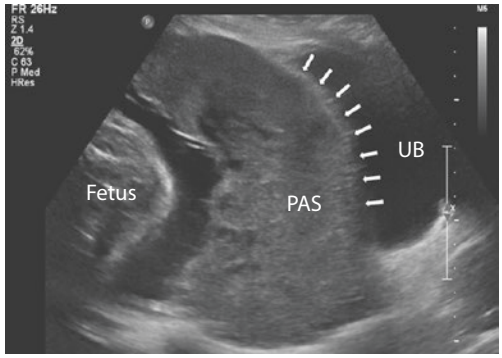


Fig. 8.9 Disruption of the urinary bladder wall. B-scan shows a disruption or loss of the hyperechogenic ligament ("line") between the uterine serosa and the urinary bladder lumen

Uterovesical Hypervascularity

In many cases of PAS, there is pronounced neovascularisation between the uterine surface and the bladder, even in the absence of bladder infiltration by a placenta percreta (Fuchs et al. 2008). In the course of neovascularization, the vessels of the uterine serosa and the bladder wall are sonographically enhanced. A distinct color Doppler signal is found between the myometrium and the posterior wall of the bladder, indicating the high number of densely arranged, tortuous vessels in this region (Fig. 8.10). Often a multidirectional flow with aliasing effects can be detected.

Subplacental Hypervascularity

In PAS, the color Doppler shows a pronounced signal in the placental bed with increased blood flow. Multidirectional flow and aliasing effects can be seen as a sign of a high number and density of strongly tortu-

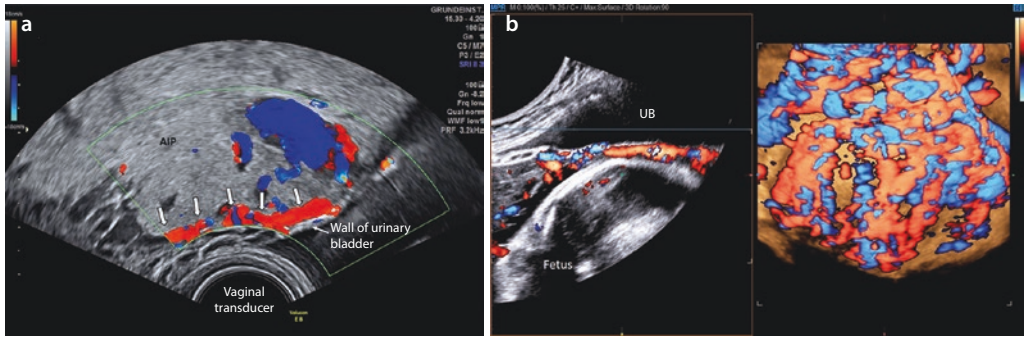


Fig. 8.10 **a, b** Uterovesical hypervascularity. **a** Sonographically distinct color Doppler signals between the myometrium and the posterior wall of the urinary bladder, indicating a high number of densely arranged, tortuous vessels in this region. Detection of multidirectional flow and aliasing effects is often successful. **b** 3D subvesical vascular bed imaging. *UB* Urinary bladder

ous vessels (Fig. 8.11). However, the assessment of "vascularization" is very subjective and depends on the experience of the examiner as well as significantly on the device setting (Tutschek et al. 2014). Especially when combined with volume (3D) imaging, color Doppler can generate impressive images of the vascular architecture (Fig. 8.12) (Shih et al. 2009; Collins et al. 2015). Prominent subplacental uterine vessels can be visualized as they progress parallel to the serosa (Resnik 2014). An own histological examination of normal placentas and placentas with PAS, published in 2012, in which the placental vascular architecture was qualitatively and quantitatively examined, confirmed the different vessel distribution and, above all, the presence of larger vessels in PAS (Chantraine et al. 2012).

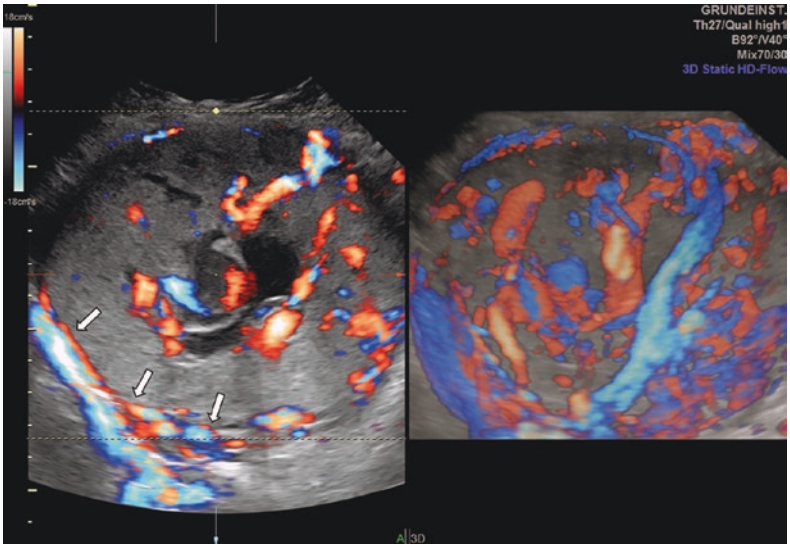


Fig. 8.11 Subplacental hypervascularity. In PAS, color Doppler shows a distinct signal in the placental bed with increased blood flow. Multidirectional flow and aliasing effects can be seen as a sign of a high number and density of strongly tortuous vessels

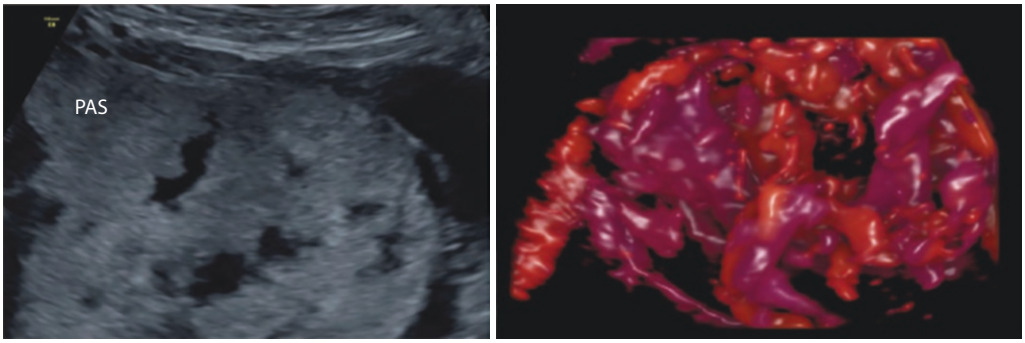


Fig. 8.12 B-scan and 3D angiomode with plastic representation of the complex vascular structure of a PAS in the area of the uterine side wall

Bridging Vessels

Bridging vessels describe the vessels that can be visualized on color Doppler that originate from the placenta and extend beyond the myometrium and uterine serosa into the urinary bladder wall or other organs. These vessels often run at right angles to the uterine surface and to the myometrial plane (Fig. 8.13). These vessels appear to directly connect the placental vessels to those of the myometrium at the implantation site (Wong et al. 2008, 2009).

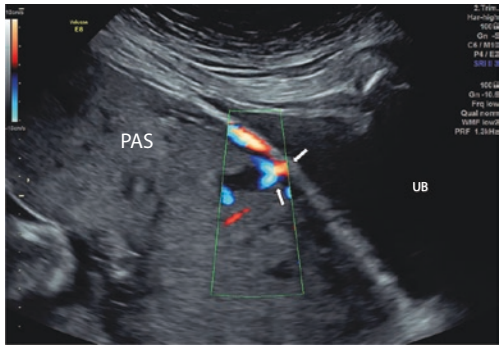


Fig. 8.13 Bridging vessels. Vessels that can be visualized on color Doppler that originate from the placenta and extend beyond the myometrium and uterine serosa into the urinary bladder (UB) wall or other organs. These often run at right angles to the myometrial plane

Vessels Feeding the Placental Lacunae: Lacunae Feeder Vessels

Another PAS sign that can be detected sonographically is the presence of vessels with a strong blood flow originating from the myometrium and feeding the placental lacunae (Fig. 8.14). Turbulence is often seen immediately after entry into the lacuna (Collins et al. 2015).

Summary Ultrasound

To the current knowledge of the published literature, none of the described ultrasound signs have been evaluated as sufficient on their own to predict PAS, with the exception of proven bladder invasion. All of the markers described should always be evaluated in combination with the risk history. However, from experience, a decrease in myometrial thickness and hypervascularization of the underlying placenta are the first important signs of PAS. Abnormalities on color Doppler have the highest detection rate for

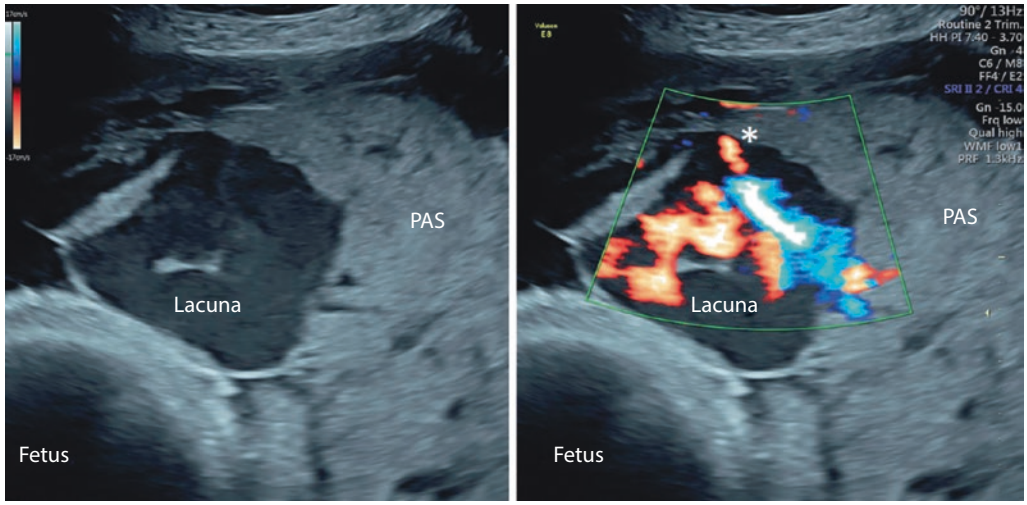


Fig. 8.14 Lacunae Feeder Vessels. Sonographic image of vessels with a strong blood flow originating from the myometrium and feeding the placental lacunae. Turbulence immediately after entry into the lacunae is common

PAS, but abnormalities at the uterus-bladder junction are the most specific. The presence of one or more ultrasound signs of PAS increases the a priori risk of PAS by a factor of 11 (Tutschek et al. 2014). In a meta-analysis of more than 3700 high-risk cases on the accuracy of sonographic diagnosis of PAS, a prepartum detection rate (all ultrasound methods) showed a sensitivity of 91% with a specificity of 97% (Woodring et al. 2011). However, in another study with a non-pre-selected patient population and blinded investigators, the sensitivity was significantly lower at 53% (Bowman et al. 2014). The highest detection rate of 91% was for color Doppler signs, followed by prominent lacunae (77%). Lower sensitivities were reported by the authors for loss of echo-deficient myometrium (66%) and abnormalities at the uterus-bladder junction (50%) (Woodring et al. 2011). Volume ultrasound in combination with color or power Doppler has also been successfully used to confirm the diagnosis of PAS (Henrich and Stupin 2011).

Currently, a prospective evaluation of IS-AIP is investigating the value of sonographic signs (Collins et al. 2016) in terms of the potential to detect PAS (Alfirevic et al. 2016).

MRI for the Diagnosis of PAS

MRI is another diagnostic method to examine pregnant women in sonographically suspected cases of PAS. Especially in cases of suspected infiltration of neighboring organs, unfavorable sonographic conditions or the rare cases of PAS at sites other than the anterior wall of the uterus, the performance of an MRI can provide valuable information for better therapy planning (Palacios Jaraquemada and Bruno 2005). As with ultrasound, the experience of the examiner is critical to diagnostic accuracy and accurate interpretation of findings on MRI (Alamo et al. 2013). Controversy exists as to whether an MRI is more accurate than an ultrasound examination for the diagnosis of PAS (D'Antonio and Bhide 2014). In a 2014 review of eight comparative studies of the use of MRI and ultrasound on 255 women with PAS, no statistically significant superiority of MRI vs. ultrasound for diagnosing PAS was detected (MRI: sensitivity 90%, specificity 88%; ultrasound: sensitivity 86%, specificity 89%) (D'Antonio et al. 2014). Hence, sonography remains the gold standard for diagnosis.

The MRI signs for PAS cited in the literature are very heterogeneous, analogous to ultrasound signs, and different descriptions are used for the same phenomena. In analogy to ultrasound signs, the IS-AIP has therefore currently developed a proposal for the standardization of MRI signs (Chabot-Lecoanet et al. 2017). In addition to the signs already known from ultrasound, placental infarcts, dark intraplacental signal bands and abnormal vascularization in the placental bed are also listed here (◘ Table 8.3). The diagnostic value of these MRI signs is currently being evaluated prospectively.

Newer techniques, such as fusion imaging combining morphological information with functional parameters (blood flow imaging), may be able to achieve an even better delineation between placental bed and myometrium in the future (Morita et al. 2009).

Currently, MRI diagnostics should be used in addition to ultrasound in suspected PAS cases with unclear findings, posterior wall/sidewall placenta, suspected infiltration of the parametria or other neighboring organs, and insufficient ultrasonographic conditions (Abuhamad 2013; Berkley and Abuhamad 2013). For the clinical decision and therapy planning, the more unfavorable

Table 8.3 Compilation of a unified definition by the European Working Group on Abnormally Invasive Placenta (EW-AIP) of the MRI signs of PAS currently used in the literature. (Chabot-Lecoanet et al. 2017)

MRI signs	EW-AIP standardized proposals to unify definitions
Thinning or absence of the myometrium	Decreased or absent hypoechogenic myometrium above the placenta with <1 mm or less
Placental protrusion	Deviation of the uterine serosa from the expected area caused by abnormal protrusion of placental tissue into neighboring organs, typically the urinary bladder. The uterine serosa appears intact, but the surface is distorted
Loss of the retroplacental dark zone	Loss of the dark zone below the placental bed
Heterogeneous placenta	Heterogeneous signaling within the placental structure
Dark bands within the placenta	One or more areas of low signal intensity, nodular or rectilinear in appearance, which are in contact with the placental-myometrial junction
Placental infarcts	Blurred areas with increased signal intensity in T1 sequences
Urinary bladder disruption	Irregular presentation or interruption of the normally hypointense urinary bladder wall
Focal, expophytic excrescence	Placental tissue which breaks through the uterine serosa and can be visualized beyond it, usually seen within a filled urinary bladder
Abnormal vascularization in the placental bed	Large vessels within the placental bed with disruption of the placental-myometrial boundary layer

findings (US, MRI) should be used for decision-making (McLean et al. 2011). However, it should be left to the surgeon to adapt the surgical procedure according to the surgical site.

Laboratory Tests

Laboratory parameters such as an elevated serum alpha-fetoprotein level could be positively associated with the presence of PAS in some case series (Zelop et al. 1992; Kupferminc et al. 1993; Hung et al. 1999; Dreux et al. 2012). However, the parameter alone cannot be used to verify PAS, and inconspicuous serum levels do not exclude PAS. Other parameters, such as angiogenesis factors, apoptosis markers, etc., are currently under review but do not yet have clinical relevance (Wehrum et al. 2011; Oztas et al. 2016; Buke et al. 2017).

8.1.7 Management and Therapy

In the following, the management of PAS cases is discussed depending on the timepoint of diagnosis and the mode of birth (see overview below) (Henrich and Braun 2013).

Checklist for Prepartum Detection of PAS (Henrich and Braun 2013; Tutschek et al. 2014)

- Specific obstetric and gynecological history with risk factors (Table 8.1), including previous operations such as C-section, curettage, other uterine surgical interventions
- Ultrasound examinations should be performed both transabdominally and transvaginally

- 2-dimensional examination by means of B-mode imaging with a half-filled bladder in order to be able to assess the placenta including possible lacunae and the lower uterine segment
- Color Doppler examination of the placenta and placental bed
- Volume (3D) ultrasound, which is particularly valuable for the documentation of findings and longitudinal examinations
- In cases of suspected diagnosis of PAS, examination for depth of infiltration and precise localization and extension is important:
  - Assessment of the main mass of the placenta and extension of the placental margins
  - Identification of the uterine area which guarantees access avoiding the placenta or transplacental child development
  - Localization of the umbilical cord insertion
- It is recommended to differentiate between anterior, lateral and posterior placenta as well as between low anterior and high anterior placenta outside the incision area in case of C-section with transverse isthmic uterotomy as well as to assess cervical invasion
- Special attention in relation to the urinary bladder should be paid to the vessels between the bladder and uterus, in addition to infiltration

Possible Procedure in case of Antenatal Diagnosis

Antepartum Management

Within the framework of antepartum management, an interdisciplinary presentation and consultation of the pregnant woman by obstetricians, gynecologists, neonatologists, radiologists, urologists and anesthetists takes place. Detailed information of the patient about the planned mode of delivery and the need for evaluation of the intraoperative situs is required. Psychological support of the patient is recommended both prepartum and postpartum, as the pregnant woman is confronted with the seriousness of the disease due to possible high blood loss on the one hand and possible loss of fertility after surgery on the other hand. Preoperatively, the aim should be to achieve a maternal baseline hemoglobin value as high as possible; if necessary, oral or intravenous Fe substitution should be given. An emergency plan (24 h/7 days) with contact persons and telephone numbers is hold available. Inpatient admission is possible from the 33rd week of gestation in the event of an abnormal course, or earlier in the event of the onset of labor, a cervix length of <30 mm, bleeding, premature rupture of the membranes or poor accessibility to the clinic. Induction of lung maturation (betamethasone two times 12 mg i.m.) should be performed if there is a history of increased risk of preterm birth, preterm symptoms, or bleeding ≤34 + 0 gestational weeks. Anti-D immunoglobulin administration is performed in case of vaginal bleeding and Rh(D)-negative status.

With a median blood loss of 2.5–7.8 l (Stotler et al. 2011; Shamshirsaz et al. 2015), sufficient blood should be cross-fed prepartum and plasma, platelet concentrates, etc. should be kept in adequate supply at all times. Vaginal digital examinations, especially in cases of placenta praevia percreta should be avoided.

The timepoint of cesarean delivery in the case of a prepartum diagnosis, so far inconspicuous clinical course without bleeding, etc., should be between 34 + 0 and 35 + 0 weeks of gestation, according to studies, taking into account preterm birth and avoiding the onset of preterm labor, premature rupture of the fetal membranes (Warshak et al. 2010; Shamshirsaz et al. 2015), and accord-

ing to expert consensus between 34 + 0 and 36 + 6 weeks of gestation (Robinson and Grobman 2010; Belfort 2011; Spong et al. 2011; D'Antonio et al. 2016). The individual clinical course and the risk of a necessary unplanned delivery due to premature onset of labor should always be taken into account.

Neonatal outcome in patients with PAS appears to be independent of the depth of invasion or extent of PAS, with a significant increase in preterm births and small-for-gestational-age (SGA) infants. Here, the primary perinatal problems are those typical for gestational age (Gielchinsky et al. 2004; Seet et al. 2012).

Surgical Procedures

In case of suspected urinary bladder wall infiltration, parametric infiltration or urinary retention, a double-J ureteral stent should be applied by the urologist after peridural anesthesia (PDA). In addition, a 3-way Foley catheter should be placed if a bladder wall resection is planned.

The use of balloon catheters for temporary vessel occlusion during surgery and thus a potential reduction of blood loss is controversially discussed. In addition to studies that have demonstrated a significant reduction in blood loss (Ojala et al. 2005; Angstmann et al. 2010; Ballas et al. 2012), there are other publications that show no significant improvements with the use of balloon catheters (Dilauro et al. 2012; Salim et al. 2015; Omar et al. 2016). In addition, there are reports of complications after balloon occlusion such as dislocation, aneurysm, vessel wall dissection, rebleeding at the puncture site, etc. (Sewell et al. 2006; Greenberg et al. 2007).

The "Triple-P procedure" was developed as a conservative surgical alternative to peripartum hysterectomy for PAS (Chandraharan et al. 2012). Triple-P involves three steps: Perioperative placental localization with a transabdominal ultrasound scan to delineate the upper border of the placenta on the operating table, cross-sectional uterotomy, and delivery of the fetus above the placental margin; followed by balloon occlusion to reduce the blood supply to the myometrium by balloon catheters already placed before surgery ("pelvic devascularization") and finally, without detaching the placenta ("placental nonseparation"), performing focal resection of percreted placental areas followed by uterine wall reconstruction.

In most cases, a longitudinal laparotomy up to the navel is performed. Depending on the placental localization the access via a Pfannenstiel incision is also possible in case of planned focal resection, early gestational age or very low placental position.

It is important that the uterotomy should always be performed cranial to the placental margin, if necessary through a fundal cross-section (◘ Fig. 8.15). This is also possible by extruding the uterus with the child still inside (Wax et al. 2004).

Small focal findings with deep placental implantation can be treated by uterine wall resection with preservation of the uterus, possibly with transmural sutures (Palacios Jaraquemada et al. 2004). In the case of extensive findings, supracervical sectiohysterectomy should be performed en bloc, preferably without prior attempt at placental abruption (Lockwood and Russo-Stieglitz 2013). In case of uterocervical invasion, extirpation of the cervix will be unavoidable in many cases.

Conservative Procedure

A conservative approach with child delivery bypassing the placental attachment site and leaving the placenta in utero is also possible. Waiting for a two-stage placental delivery or later hysterectomy under hemodynamically more favorable conditions, if necessary also transfer of the patient to an appropriately qualified center, represent options in less well-supplied regions.

In the conservative procedure with leaving the placenta in utero, no manipulation of the placenta follows after birth. The

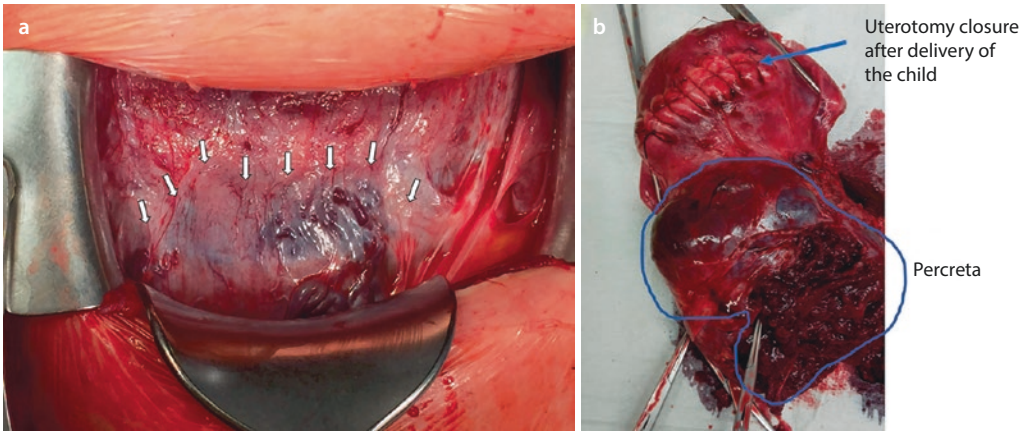


Fig. 8.15 **a** Intraoperative visualization of a large anterior wall PAS with a pronounced percreta component and visualization of numerous large-volume vessels as signs of neovascularization. **b** Hysterectomy specimen

uterotomy is closed in two layers with the administration of the usual uterotonics. In principle, if the placenta is left in place, a latency period of several weeks is possible before placental detachment and delivery may occur (O'Brien et al. 1996; Henrich et al. 2002). Success rates for a conservative approach are 40–60% (Timmermans et al. 2007). In the case of postoperative hemoglobin-related bleeding after the placenta has been left in place, hemostasis can be achieved by means of uterine embolisation or by inserting a balloon catheter into the internal iliac artery (Bodner et al. 2006).

In a study of the conservative approach in cases of PAS in a total of 167 women, the uterus was preserved in 131 women (78%), 18 women (11%) required secondary hysterectomy within 24 h, and a further 18 women (11%) underwent secondary hysterectomy after a median of 39 days (Sentilhes et al. 2010a). Significant morbidity with sepsis, vesicouterine fistulae and/or uterine necrosis was seen in ten women (6%). The median duration of placental resorption was 13.5 weeks (range 4–60 weeks) (Sentilhes et al. 2010a). In another study of 434 patients and different conservative treatment regimens, severe vaginal bleeding was reported in 53%, sepsis occurred in 6% of patients, and the mortality rate was 0.3% (Steins Bisschop et al. 2011).

Most women can become pregnant again with the conservative, uterus-preserving procedure, but the rate of recurrence of PAS is 29% (Sentilhes et al. 2010b). In our own collective, two women with PAS and extensive segmental resection each gave birth to another child by resectio after an uncomplicated pregnancy without PAS. Two other women with conservative management, retention of the placenta and subsequent spontaneous placental birth after 24 and 48 months, gave birth to two more children, each without PAS, by uterus-preserving C-section (Henrich et al. unpublished).

If a two-stage hysterectomy has to be performed due to bleeding, surgical morbidity and the need for transfusions are significantly lower due to the partial involution of the uterus (Comstock 2005). The main risk factor of conservative management is infection, in up to 25% of cases (Timmermans et al. 2007).

If there is increased bleeding after intraoperative manual placental abruption during C-section or after spontaneous delivery, further surgical measures (intracavitary stitching, balloon tamponade, uterine compression sutures, e.g. according to B-Lynch,

etc.) or a combination of balloon and compression sutures (sandwich technique) are necessary.

Possible Procedure in case of Intrapartum Diagnosis

If PAS is only diagnosed intrapartum after spontaneous delivery with no placental separation and increased bleeding, manual placental separation with post-curettage may be indicated. Peripartum curettage after vaginal delivery should be performed under ultrasound guidance to avoid perforation of the uterine wall or leaving placental remnants behind (Krapp et al. 2007). A conservative procedure with leaving parts of the placenta in utero may be necessary if not all ingrown parts of the placenta can be removed from the uterine wall ("piece meal removal"). If there is no increased bleeding, this is also possible after vaginal birth (Timmermans et al. 2007). The placenta or parts of the placenta can be removed spontaneously after a period of time that can last up to several weeks, or in a second curettage. The prerequisite for this is close ambulatory monitoring of infection and coagulation parameters and, above all, good compliance on the part of the patient. Continuous antibiotic prophylaxis should be considered on an individual basis.

Both embolization of the uterine arteries and the application of an intrauterine balloon tamponade (e.g. Bakri balloon) or the use of Celox tamponades are additive therapy options in the case of persistent heavy bleeding from the placental bed (Schmid et al. 2013). Rapid and consistent hemostaseologic stabilization with correction of loss and impending consumption coagulopathy are prerequisites for uterine preservation. In case of unstoppable bleeding, hysterectomy is indicated as the last resort.

8.2 Placenta Praevia

Julia Knabl and Franz Kainer

8.2.1 Terminology

The term placenta praevia ("prae viam", before the way) defines a placenta after 20 + 0 weeks of gestation with an implantation site in the lower uterine segment and thus closely related to the internal cervical os.

A widely used historical classification of placenta praevia is based on the results of the clinical examination: The dilated cervix was inspected and manually palpated to describe the relationship of the placental margin to the internal cervical os (Table 8.4) (Dashe 2013).

However, since manual examination has been replaced by sonography, it is recommended to use a terminology which is oriented towards the sonographic findings. In addition, the distinction between placenta praevia partialis and marginalis is often difficult in the clinical examination.

Table 8.4 Classification of placenta praevia according to clinical criteria

Type	Clinical criteria
Placenta praevia completa	Placenta completely covers the internal cervical os
Placenta praevia partialis	Placenta partially covers the internal cervical os of the dilated cervix
Placenta praevia marginalis	Placenta touches the edge of the internal cervical os
Low-lying placenta	Placenta does not reach the internal cervical os

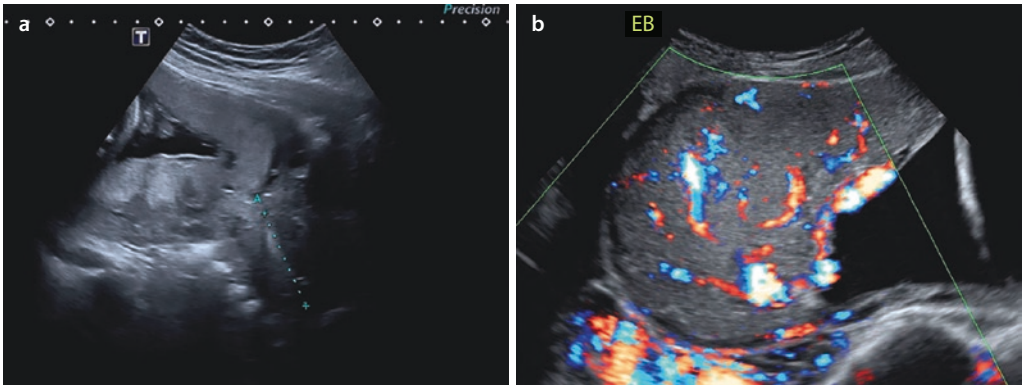


Fig. 8.16 Placenta praevia. **a** Sagittal section, distance A indicates cervical length (B-scan). **b** Sagital section with increased vascularization, covering the entire anterior wall (B-scan with color Doppler)

Because of this difficulty of delineation and especially the lack of clinical relevance (Oppenheimer et al. 1991), the designations placenta praevia partialis and marginalis were abandoned and replaced by the following classification.

Placenta praevia (Fig. 8.16) refers to all cases in which the internal cervical os is covered by placental tissue. If placental tissue is present only in the vicinity of the internal cervical os, it is referred to as a low-lying placenta, whereby the distance to the internal cervical os is to be indicated (Reddy et al. 2014).

8.2.2 Morbidity and Mortality

Maternal morbidity in placenta praevia constellations is determined by the extent of blood loss during and after delivery. Blood loss depends to a large extent on the position of the placenta (anterior or posterior placenta) and on the presence of an additional invasion defect (placenta increta, percreta). Furthermore, the lower uterine segment contracts worse. These points explain the increased need for blood products and plasma substitutes, the greater number of hysterectomies and the longer stays in the intensive care unit (Gibbins et al. 2017).

Infant morbidity, in addition to prematurity, is determined by fetal blood loss during delivery. Children of patients with placenta praevia have a fivefold higher risk of premature delivery, need for intensive care, and death compared to children of mothers with normal placental location (Vahanian et al. 2015).

The rate of malformations appears to be slightly increased (OR 1.6) (Kancherla et al. 2015).

Whether a low-lying placenta impairs fetal growth is controversial: Data in the literature vary from a moderate association between placenta praevia and FGR ("fetal growth restriction") (Ananth et al. 2001; Rosenberg et al. 2011), to no association (Norgaard et al. 2012; Yeniel et al. 2012; Lal and Hibbard 2015).

A recent cross-sectional study of the Finnish Birth Registry found a doubling of the probability of having a small-for-gestational-age child with placenta praevia in multiparous women after adjusting for all known risk factors; they found no change in primiparas (Raisanen et al. 2014).

8.2.3 Etiology and Risk Factors

The incidence of placenta praevia is estimated to be one in 200 pregnancies, but it varies worldwide (0.6–4/200 pregnancies) (Cresswell et al. 2013). The main reason for the increasing incidence of placenta praevia is particularly the increase in incisional deliveries, C-sections (Faiz and Ananth 2003).

The reasons for the strongly varying prevalence are the different ways of diagnosis (clinical diagnosis vs. sonographic diagnosis), the different timepoints of diagnosis as well as the non-comparable study collectives.

In the middle of the second trimester, the placenta sits close to the cervix in 2% of all pregnancies (Becker et al. 2001). The so-called placental migration (the placental location normalizes in the course of pregnancy) is due to the growth of the placenta-free uterine wall. Persistence into late pregnancy depends on the degree of overlap at the beginning of pregnancy. In 90% of placentas with low overlap, the location normalizes. There is also an increased risk of inadequate placental migration with a previous cesarean section (Dashe et al. 2002).

Histopathological studies of placental biopsies show increased invasion and increased numbers of trophoblast giant cells in decidua and myometrium in placenta praevia and increased physiological remodeling of spiral arterioles (Biswas et al. 1999).

In the lower uterine segment, decreased blood flow is thought to occur during the first trimester due to the higher percentage of connective tissue and lower percentage of muscle. This local hypoxia and the thinner endometrium could cause the increased trophoblast invasion and the increased physiological remodeling of the spiral arteries (Hasegawa et al. 2011).

This increased infiltration of the trophoblast may increase perfusion and oxygenation of the placenta. Thus, preeclampsia and placenta praevia have opposing pathophysiological mechanisms: preeclampsia is classically due to decreased perfusion and oxygenation of the placenta (Ying et al. 2016). This effect may be one reason for the reduced incidence of preeclampsia in patients with placenta praevia (Ananth et al. 1997; Ying et al. 2016). Even an overall reduction in arterial blood pressure has been observed in patients with low-lying placenta and low-lying umbilical cord (Hasegawa et al. 2011).

Histopathologically, there are also frequent hemorrhages into the myometrium with an acute inflammatory reaction in the myometrium and the fetomaternal vessels.

The incidence of placenta praevia is associated with the following risk factors (Faiz and Ananth 2003):

- Uterine surgeries: The likelihood of developing placenta praevia increases with the rate of cesarean sections, abortions, surgical terminations and other uterine surgeries (Ananth et al. 1997; Getahun et al. 2006). Here, scarring and damage to endometrium and myometrium after uterine surgeries may be causative factors. The increasing incidence of placenta praevia is mainly due to the increase in incisional deliveries (Faiz and Ananth 2003). A single history of cesarean section increases the prevalence of placenta praevia from 0.5% to 5% (Solheim et al. 2011).
- Increased maternal age and multiparity: The cause is assumed to be arteriosclerotic changes in the maternal vessels, which cause reduced perfusion of the placenta and thus placental infarctions. Thus, a widened placental surface is necessary to maintain an adequate blood flow. This in turn brings with it an increased likelihood of overlapping of the lower uterine segments (Williams and Mittendorf 1993).

  The same applies to the risk factor multiparity: It is suspected that scarring

exists in the area of placental adhesion sites of a previous pregnancy, which results in reduced perfusion of the new placenta. This could also lead to an increased placental adhesion area with overlapping of the inner cervical os (Naeye 1978).

- Smoking and drug abuse, especially cocaine: The vasoactive effects of nicotine and chronic hypoxia from carbon monoxide alter the uterine vascular bed and lead to reduced perfusion and compensatory enlargement of the placenta, which is associated with a higher likelihood of overlapping the internal cervical os (Williams et al. 1991). Thus, increased surface area macroscopically (Christianson 1979) and microscopically (Pfarrer et al. 1999) due to excessive angiogenesis in placentas of smokers has long been described to compensate for chronic hypoxia resulting from carbon monoxide. Nevertheless, smoking damages placental function and blood flow (vasoconstriction, infarcts) to such an extent (Zdravkovic et al. 2005) that the rate of growth-retarded fetuses and intrauterine fetal death is significantly increased.
  Cocaine-induced catecholamine-mediated vasoconstriction appears to act in a similar manner: Decreased blood flow to the uteroplacental vessels results in enlargement of the placenta and thus coverage of the internal cervical os (Moore et al. 1986; Handler et al. 1994; Macones et al. 1997).
- Assisted reproduction: In vitro fertilization (IVF) also increases the risk of placenta praevia (Sazonova et al. 2011a,b; Ginstrom Ernstad et al. 2016). Whether intrauterine location of embryo transfer plays a role is controversial (Romundstad et al. 2006; Healy et al. 2010). The increased risk of placenta praevia, particularly after blastocyst transfer, suggests that in vitro culture alters early embryo development and implantation. Furthermore, the influence of epigenetic mechanisms is discussed, e.g. DNA methylation, which plays a decisive role in trophoblast invasion (Nelissen et al. 2011).
- Multiples: The probability of placenta praevia is 1.4 times higher in multiples compared to singletons with comparable risk factors. In multiples, the increased placental mass increases the probability of placenta praevia (Ananth et al. 2003).

Special Case Placenta Praevia Accreta

Five percent of all placentas located anterior to the internal cervical os detach inadequately postpartum.

The term placenta accreta is used on the one hand as an umbrella term for the individual degrees of invasion, on the other hand placenta accreta describes an infiltration of the trophoblast into the decidua basalis. Placenta increta usually refers to deep infiltration into the myometrium, whereas placenta percreta means that the infiltration extends into the serosa or adjacent organs (► Sect. 8.1.4).

In particular, a previous cesarean section and placenta praevia are strong risk factors. The incidence of placenta accreta is also increasing due to the rising rate of cesarean sections. A patient with two (three) previous sections has a 40% (60%) risk of placenta praevia accreta if the placenta is inserted anterior to the cervix, but only 0.1% if the placenta is normally located (Silver et al. 2006a, b; Jauniaux and Jurkovic 2012).

The explosiveness of this topic increases continuously with the rising rate of C-sections. This causes the increasing incidence of placenta praevia and placenta praevia accreta and leads to an increased maternal mortality.

8.2.4 Diagnostics and Management

The gold standard for the diagnosis of placenta praevia is the transabdominal and transvaginal ultrasound. Especially the transvaginal examination only allows in many cases the exact localization of the placenta and its relation to the internal cervical os. Careful transvaginal ultrasonography does not provoke bleeding and is considered a safe method (Smith et al. 1997). 3D techniques can facilitate orientation and accurate visualization of localization in multiplanar mode (Palacios-Jaraquemada 2013). With high negative predictive power, placenta praevia can be excluded by ultrasound at any gestational age. However, in pregnancies below 16 + 0 to 20 + 0 weeks of gestation, the diagnosis is incorrectly made too frequently. The degree of invasion of the myometrium can also be well assessed by ultrasound (Chalubinski et al. 2013) (▶ Sects. 8.1.6 and 9.1).

Clinical Management

The following pragmatic approach applies to asymptomatic women without bleeding (Reddy et al. 2014):

The placental location should be considered normal if the placental margin is >2 cm from the internal cervical os in pregnancies from 16 + 0 weeks gestation. If the placental margin is <2 cm from the internal cervical os but does not cover the internal cervical os, the placenta should be classified as low-sitting; in this case, progress ultrasonography is recommended at 28 + 0 to 32 + 0 weeks of gestation.

When the placental margin covers the internal cervical os, the placenta is called placenta praevia and sonographic follow-up should be done every 2 weeks.

The following additional sonographic findings should be noted:

- Precise localization: The position of the placenta (starting from the posterior or anterior wall) and, in the case of pathological findings, the distance to the internal cervical os should be indicated in every examination. These additional details are crucial for clinical management. They provide information on the optimal surgical approach in advance. In addition, in the case of an anterior wall placenta and previous surgeries in the area of the anterior wall of the uterus (e.g. cesarean section), the probability of a placenta praevia accreta is increased.
- Degree of invasion: Classic signs of invasion include loss of the hypoechogenic retroplacental border zone between the placenta and uterus, increased placental confluent lacunae, aberrant placental vessels, and protrusion of the placenta into the urinary bladder (▶ Sect. 8.2.3) (Guy et al. 1990; Chalubinski et al. 2013; Reddy et al. 2014).
- Cervical length: Several studies have shown that a cervical length of >3 cm significantly reduces the likelihood of vaginal bleeding and/or emergency cesarean section (Stafford et al. 2010; Sekiguchi et al. 2015).
- Thickness of the placental margin: The thickness of the placental margin over the internal cervical os (thickness of >1 cm) also appears to be a good predictor of vaginal bleeding (Ghourab 2001; Saitoh et al. 2002).
- Assessment of umbilical cord insertion, vasa praevia: Furthermore, vasa praevia (▶ Sect. 8.3.2) should be excluded with color and/or pulsed Doppler. These are found more frequently when a formerly low-lying or praevia placenta has been diagnosed in the mid-trimester. Because of the extremely increased fetal mortality, this diagnosis should definitely be made prenatally. Eccentric cord insertion is also significantly more common in placentas inserted in the lower uterine segment (Hasegawa et al. 2011).

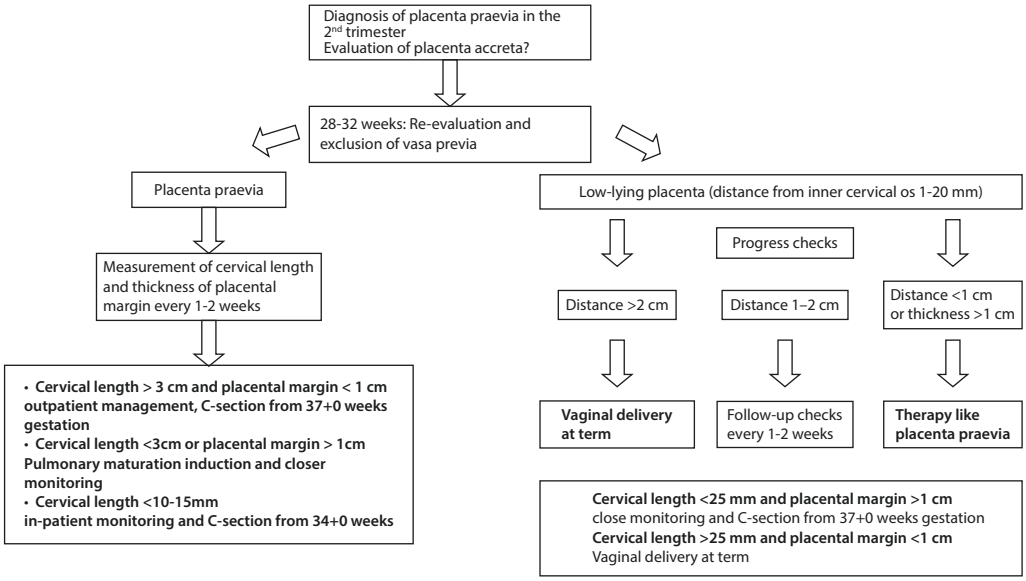


Fig. 8.17 Management algorithm for placenta praevia and low-lying placenta (without procedure for placenta accreta or vasa praevia)

- Fetal status and fetal position: Fetal positional abnormalities are increased in placenta praevia and low-lying placenta, this is particularly important preoperatively. Fetal status testing (Doppler/CTG) is also advisable, as the rate of FGR is controversial (▶ Sect. 8.2.2) and umbilical cord alterations may be more commonly associated with compromised care.

An algorithm for clinical management (modified from Vintzileos et al. 2015) is shown in Fig. 8.17.

8.2.5 Operational Procedure

Careful planning in advance of the surgery is essential to ensure that the necessary resources are readily available in case of doubt. An experienced team (surgeon, anesthetist and neonatologist) and sufficient blood products are important in this context (Palacios-Jaraquemada 2013).

Emergency Situation

In cases of life-threatening maternal hemorrhage, the first priority is rapid fetal delivery and simultaneous hemodynamic stabilization. Emergency hysterectomy prior to hemodynamic stabilization should be avoided as this procedure is associated with further blood loss of 2–3 l (Lier et al. 2016).

Placenta Praevia, Originating from the Posterior Wall

This procedure differs only slightly from a regular cesarean section. It is possible that there may be increased bleeding after placental separation due to the reduced contractility of the lower uterine segment. Otherwise, no major complications are to be expected with this procedure.

Placenta Praevia, Originating from the Anterior Wall

With anterior wall placenta praevia, blood loss (OR 2.97; 95% CI: 1.64–5.37), use of mass transfusion (OR 3.31; 95% CI: 1.33–8.26) and number of hysterectomies required (OR 3.47; 95% CI: 1.39–8.68) are significantly increased (Jang et al. 2011). It should also be noted that anterior placenta praevia and previous cesarean section significantly increase the risk for the presence of placenta accreta (Marshall et al. 2011).

If possible, transplacental delivery of the child should be avoided, as this provokes maternal and fetal bleeding. This is a particular surgical challenge in the case of placenta praevia originating from the anterior wall. The surgeon is advised to determine the exact placental localization and fetal position again by sonography prior to cesarean section in order to select the optimal access route.

A method first described by Ward in 2003 aims to avoid damaging the placenta before fetal delivery. For this purpose, myometrium and placenta are dissected manually and the placenta is partially detached until the fetal membranes can be palpated. Then, amniotomy can be performed after a turning maneuver, if necessary. The child is delivered in the area without the placenta via uterotomy (Ward 2003). If the uterotomy has to be placed over the placenta, it helps to minimize blood loss if clamps are placed beforehand (Fig. 8.18).

Atony Risk

After fetal delivery, before the placenta is separated, uterotonics are administered. After the placenta is detached, manual compression of the uterus may be necessary to stop bleeding. If this is not sufficient, compression by the intrauterine Bakry balloon, compression sutures according to Cho (Cho et al. 2000) or placental sutures according to Kainer may be considered (Kainer et al. 2003). The combination of balloon and compression sutures is also possible. Classical compression sutures such as B-Lynch or Pereira, on the other hand, are less suitable, as they mainly com-

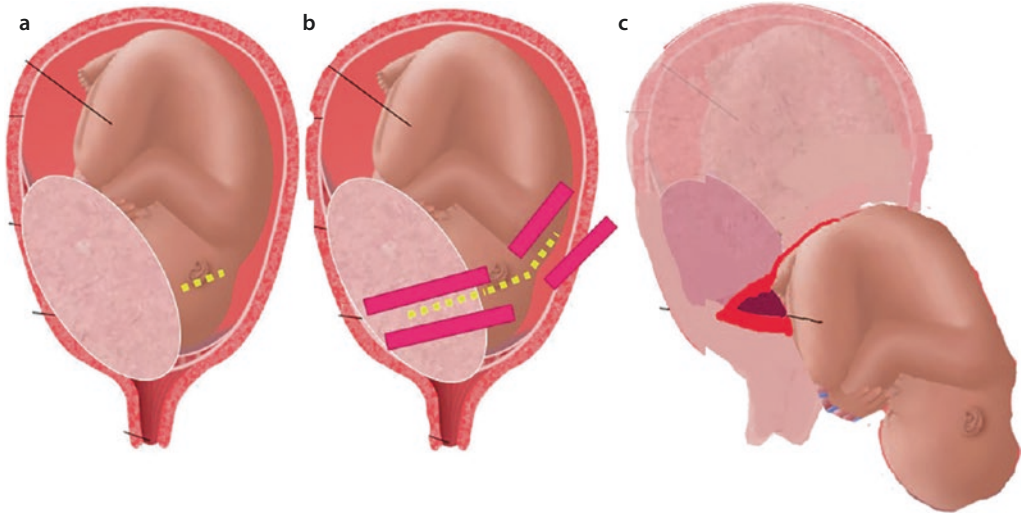


Fig. 8.18 Surgical technique for placenta praevia at the anterior wall with clamps. **a** Clamps are placed in the placenta-free area after uterotomy; **b** Clamps are pushed over the placenta and then this area is cut; **c** the child can now be developed over the sufficiently large uterotomy without injury to the placenta. *Pink parallel stripes* Clamps, *yellow dots* Uterotomy

press the corpus uteri. A local tamponade with a procoagulant effect (e.g. Caelox, off-label use) is another alternative.

8.2.6 Summary

- The incidence of placenta praevia is increasing due to the increase in incisional deliveries.
- Maternal morbidity is determined by the extent of blood loss during and after delivery, while infant morbidity is determined by blood loss during delivery in addition to prematurity.
- The gold standard for the diagnosis of placenta praevia is transabdominal and transvaginal ultrasound.
- To be assessed are: Localization, invasion, cervical length, thickness of the placental margin, umbilical cord attachment and vasa praevia, as well as fetal condition and position.
- The aim is to perform the planned cesarean section as soon as possible.
- In particular, placenta praevia, which originates from the anterior wall, carries a significantly increased risk for mother and child.
- The transplacental delivery of the child should be avoided.
- Postpartum, attention must be paid to adequate atony management.

8.3 Umbilical Cord Insertion, Variations and Vasa Praevia

Renaldo Faber

8.3.1 Umbilical Cord Insertion, Velamentous Cord Insertion

Normally, the umbilical cord inserts clearly in the region of the chorionic plate of the placenta as a central or lateral insertion (Fig. 8.19). Even the marginal insertion (Fig. 8.20) represents a deviation, since there is a closer relationship to the fetal

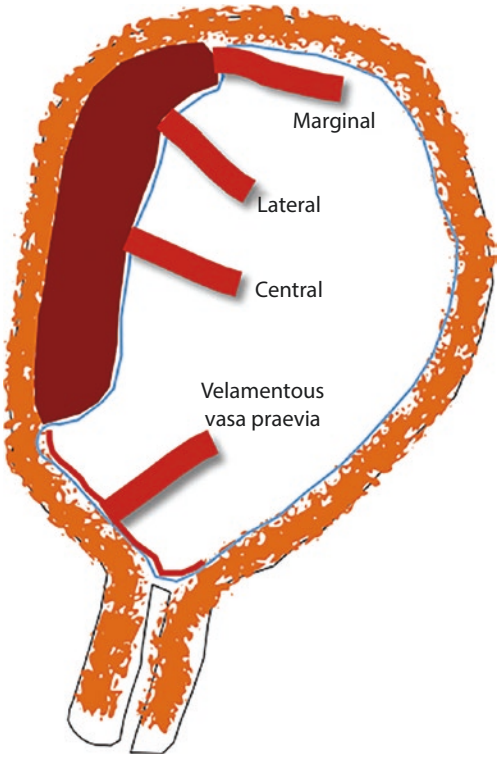


Fig. 8.19 Schematic representation of normal umbilical cord insertion, variations and vasa praevia

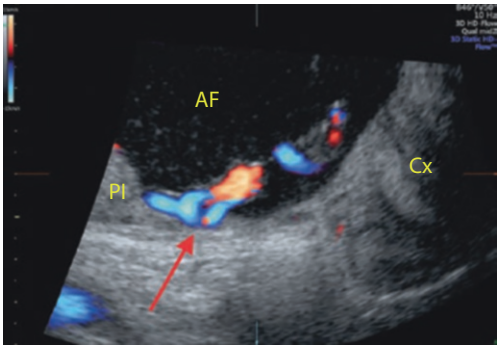


Fig. 8.20 Vaginal ultrasonography and color Doppler of the lower uterine segment showing the cervix (*Cx*), amniotic fluid (*AF*), lower pole of a posterior placenta (*Pl*), and marginal umbilical cord insertion (*red arrow*) at 21 weeks gestation

membranes and the protection of the vessels by the placenta is limited. Intrapartum, alterations of the umbilical vessels may occur with disturbance of the fetoplacental circulation (CTG changes). If the umbilical cord inserts directly at the fetal membranes, this is called a velamentous cord insertion (Fig. 8.21). In principle, a marginal insertion should be considered like a velamentous insertion, in which the vessels of the umbilical cord are no longer protected by the placenta. The length of the velamentous vessel route also plays a role in the extent of the circulatory disturbances. The longer the velamentous route is, the more frequently disturbances must be expected. We therefore recommend documenting the length, because the development of FGR can be expected more frequently with a very long course.

The overall incidence of velamentous cord insertion in singleton pregnancies is reported to be 1:100 (Sepulveda et al. 2003; Hasegawa et al. 2006a), but according to our own studies it occurs much less frequently in the "lower uterine segment" with an incidence of 1:400. In twins and higher-grade multiples, the incidence is dramatically higher at 1:4 (Fig. 8.22). In addition to other parameters, multiple births should be considered as a risk factor for the occurrence of pathological insertions (see following overview).

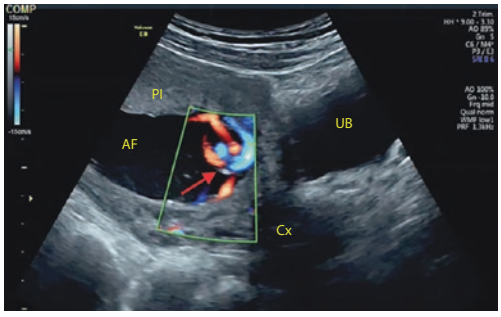


Fig. 8.21 Vaginal ultrasonography and color Doppler of the lower uterine segment showing the urinary bladder (*UB*), cervix (*Cx*), amniotic fluid (*AF*), anterior placenta (*Pl*), and velamentous cord insertion just anterior to the internal cervical os (*red arrow*)

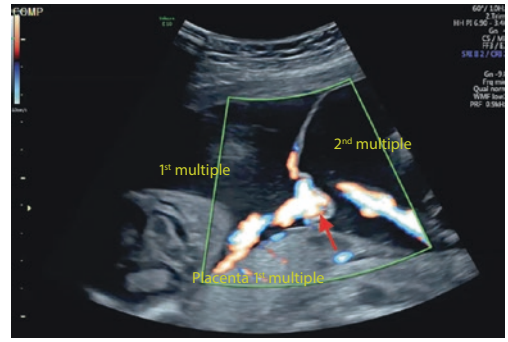


Fig. 8.22 Abdominal sonography and color Doppler showing the amniotic cavities of dichorial twins; velamentous cord insertion of the 1st multiple in the partition wall (*red arrow*) and additional velamentous vessels in the 19th week of gestation

Diagnosis is straightforward by using color Doppler to look for the placental cord in the first or second trimester (Figs. 8.20 and 8.21).

The assignment to the lower-middle-upper third of the uterus is important because velamentous cord insertion in the lower uterine segment is significantly more often associated with variable decelerations in the CTG sub partu and significantly more often leads to the need of an emergency C-section (Hasegawa et al. 2006a, b). Therefore, in addition to passive management of the placental period, clinical recommendations should point to an obstetric clinic that provides appropriate management (Table 8.5). The significance of pathological umbilical cord insertions for perinatal outcome in multiples has not been adequately studied, nor has it been considered in major management studies (Barrett et al. 2013). However, as our example shows (Fig. 8.22), this should definitely be done, especially in twins, where birth management (vaginal vs. C-section) plays a key role.

The velamentous insertion of the umbilical cord in the lower uterine segment is, among other factors, the most important risk factor for the development of vasa praevia (see following overview).

Table 8.5 Recommended management of velamentous cord insertion and vasa praevia

	Velamentous cord insertion in the middle/upper third	Velamentous cord insertion in the lower third	Vasa Praevia
Consulting	Information	Risk pregnancy	High risk
Monitoring	According to Maternity Directive	According to Maternity Directive	Intensive care, inpatient from 30–34 weeks gestation
Fetal lung maturity	–	–	30–32 weeks gestation
Maternity hospital	Individual	Level 2	Level 3
Date of delivery	At term	At term	34/35 weeks gestation
Delivery mode	Spontaneous vaginal	Spontaneous vaginal, often emergency C-section	Elective C-section
Placental period	Passive	Passive	–

Risk Factors for the Development of Velamentous Cord Insertion and Vasa Praevia

- Multiples
- Pregnancy after IVF
- Low reaching placenta
- Retracted placenta praevia
- Bipartite placenta
- Velamentous insertion of the umbilical cord in the lower uterine segment

8.3.2 Vasa Praevia

Vasa praevia are velamentous vessels which directly run over the internal cervical os or in the immediate vicinity (1–3 cm) of the internal os of the cervix. This is also the reason for their clinical relevance, as very severe fetoplacental bleeding may occur after rupture of the membranes and rupture of these vessels. The incidence of vasa praevia is reported in the literature (Hasegawa et al. 2006a; Oyelese et al. 2004) to be 1:2500. In our own unselected collective (unpublished), the incidence is 1:1300.

The risk factors for the development correspond to those of velamentous cord insertion in the lower uterine segment (overview "Risk factors for the development of velamentous cord insertion and vasa praevia"). Another very important risk factor is the frequent extreme thinning of a placenta praevia in the area of the internal os of the cervix in the course of pregnancy. The chorial vessels remain and the placenta appears as bipartite.

A former placenta praevia totalis becomes a bipartite placenta with vasa praevia (Fig. 8.23a–c).

The diagnosis is made by a combination of abdominal and vaginal sonography and the use of color Doppler and spectral Doppler sonography, preferably in the second trimester (20–22 weeks gestation). Already at the time of the 3rd ultrasound

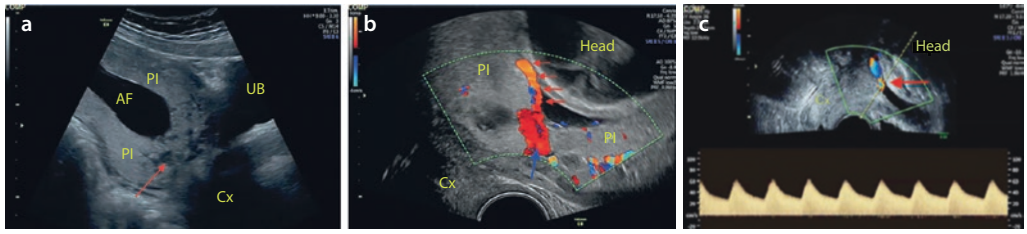


Fig. 8.23 **a**–**c** Representation of the same pregnancy at different time points. **a** Abdominal sonography at 14 weeks gestation showing maternal bladder (*UB*), cervix (*Cx*), amniotic fluid (*AF*) and total placenta praevia (*Pl*); lacunae (*red arrow*). **b** Vaginal sonography and color Doppler at 21 weeks gestation showing the cervix, placenta, and fetal head; large lacunae at the internal cervical os (*blue arrow*) and chorial vessel (*red arrows*). **c** Vaginal sonography and spectral Doppler in the 31st week of gestation showing the cervix, amniotic fluid and fetal head; chorial vessel left behind as vasa praevia (*red arrow*), placenta can no longer be visualized

screening (30–32 weeks gestation) or later in the context of birth planning, the diagnosis may be difficult or impossible (Cipriano et al. 2010).

The importance of prenatal diagnosis arises on the one hand from the high perinatal mortality of approximately 10–50% if vasa praevia remain undetected and no elective C-section is performed (Hasegawa et al. 2006b; Oyelese et al. 2004; Gagnon et al. 2009). On the other hand, survival is extremely improved (97–99%) when vasa praevia are recognized and an adapted procedure is performed (Swank et al. 2016; Catanzarite et al. 2016).

The evidence from the literature (Cipriano et al. 2010; Swank et al. 2016; Oyelese et al. 1998; Ruiter et al. 2015) and our own experience lead us to conclude that vasa praevia can only be comprehensively diagnosed if the lower uterine segment is carefully examined and the following points are always observed:

- Risk factors for the development (overview "Risk factors for the development of velamentous cord insertion and vasa praevia"),
- Diagnosis and documentation of placental cord insertion and its assignment to the uterus in the first or second trimester,
- Chorial thinning in placenta praevia.

For the diagnosis and management of vasa praevia, so far there are only individual guidelines ("clinical practice guideline") (Gagnon et al. 2009), but studies and recommendations on management become increasingly available (Swank et al. 2016; Society for Maternal-Fetal Medicine [SMFM] Publications Committee et al. 2015; Bronsteen et al. 2013; Robinson and Grobman 2011). These are consistent with our experience in Leipzig in recent years (Table 8.5). In individual cases, the management can deviate from the guidelines if certain conditions are met (e.g. short distance to the maternity hospital, high compliance of the pregnant woman, undisturbed course of pregnancy, etc.). Nevertheless, the evidence for these measures is not very high and further studies should keep this in mind.

8.4 Premature Placental Abruption

Jan Pauluschke-Fröhlich Karl-Oliver Kagan, and Harald Abele

The much-discussed phrase: "Birth is the most dangerous hour in a person's life" is put into perspective in view of the logistical and associated medical possibilities of a

modern perinatal center. Even in very difficult obstetric situations, this center is able to provide optimal care for mother and child. In view of the relevant statistics, it is therefore incomparably more dangerous to participate in road traffic with one's children than to be cared for in a perinatal center during pregnancy and childbirth. Nevertheless, there are events which – if experienced – are to be feared, especially since they require immediate decisive interdisciplinary action and the prognosis for mother and child depends above all on the experience and knowledge of the obstetric team. A particular challenge is certainly premature placental abruption, which can occur during pregnancy as well as intrapartum. In addition to endangering the pregnant woman, it is associated with a high morbidity and mortality of the affected children. Ultimately, 10% of all premature births and 10–20% of all infant perinatal deaths in the industrialized world are attributable to premature placental abruption (Tikkanen 2011).

8.4.1 Incidence and Risk Factors

The incidence of premature placental abruption is 0.5–1.0% of all pregnancies (Ananth et al. 1999, 2005; Toivonen et al. 2002; Downes et al. 2017). Cross-sectional analyses in the USA, Canada and some other countries show an increase in incidence – presumably due to an increase in pregnant women with risk factors (Table 8.6) or due to changes in case number determination (Ananth et al. 2015). Individual study groups report a slight clustering in male fetuses (Aliyu et al. 2012). Important for differential diagnosis are vaginal bleeding in placenta praevia (painless bleeding, typical position of the placenta to the internal cervical os, previous findings), marginal sinus bleeding, bleeding during an opening of the cervix, cervical polyps, con-

Table 8.6 Selected risk factors for premature placental abruption. The number of plus signs indicates the risk weighting of the individual risk factors

Risk factor	Risk weighting
Condition after cesarean section	+
Condition after premature abruption of the placenta	++++
Condition after stillbirth	++
Placental insufficiency (FGR, SGA)	++
Multiple pregnancy	++
Hypertensive pregnancy disease	
– chronic hypertension	++
– preeclampsia	++
– eclampsia	+++
Premature rupture of membranes	++
Thrombophilia	++
Bleeding in early pregnancy	+
Nicotine abuse, alcohol abuse	++
Cocaine abuse	+++
Intrauterine interventions	
– amniocentesis	+
– chorionic villus sampling	+
– laser therapy for TTTS	+++
Hydramnion	++
Uterine anomaly	+
Trauma (also minor trauma)	+++
Bronchial asthma	+
Maternal age >35 years	+

FGR fetal growth restriction; *SGA* small for gestational age; *TTTS* twin-to-twin transfusion syndrome

(According to Ghaheh et al. 2013; Gelaye et al. 2016; Lanna et al. 2017; Cheng et al. 2012; Mendola et al. 2013; Aliyu et al. 2011; Gul et al. 2016; Khattak et al. 2012; Markhus et al. 2011; Minna et al. 2011; Pariente et al. 2011)

tact bleeding or bleeding in the context of a uterine rupture. If a diagnosis of premature placental abruption is made, the risk for preterm delivery increases four to six times. The risk for stillbirth increases 8.9- to 12-fold (Ananth et al. 1999; Ananth and Wilcox 2001). The risk of maternal death peripartum increases sevenfold with premature placental abruption compared with the overall maternal mortality rate.

8.4.2 Definition

Premature placental abruption describes a detachment of the placenta before delivery of the child from the 21st week of pregnancy (SSW) onwards. A retroplacental hematoma of varying size or extent forms in the decidual layer. In the course of the detachment process, a partial placental abruption may develop into a complete abruption with considerable consequences for mother and child. The clinical spectrum and the consequences for mother and child are correspondingly large. Detachment of more than half of the placenta is often associated with intrauterine fetal death (IUFD) (Ananth et al. 1999). Loss coagulopathy forms as the bleeding progresses, which is then complicated by disseminated intravascular coagulopathy (DIC). In the context of coagulopathies, there is an unrestrained consumption of coagulation factors, opening the door to further complications (atony, uterine bleeding, need for hysterectomy, etc.).

8.4.3 Etiology

Immunological, ischemic, and/or inflammatory stimuli are thought to be responsible for the onset of premature placental abruption, leading to rupture of decidual arteries in the basal layer, resulting in the buildup of the hematoma between the uterine wall and placenta that is typical of placental abruption (Ananth et al. 2006a; Matsuda et al. 2011; Tikkanen 2010). The leaking blood spreads into the decidual layer and dislodges the placenta from its adhesion with the uterus (dissection). Depending on how far this process progresses, the exchange of gases and nutrients between mother and fetus – i.e. placental function – is permanently disrupted. When a critical exchange area is reached, the lost placental function can no longer be compensated for, and the fetus is undersupplied. In addition, the progressive blood loss endangers the health of the mother.

The development of premature placental abruption can have various causes. Trauma (abdominal trauma, fall, minor trauma, etc.) leads to shear forces between the placenta and the uterine wall, which can cause premature placental abruption (Ananth et al. 2006a, b). The risk of premature placental abruption is increased even with minor trauma (Cheng et al. 2012). On the other hand, uterine scarring (e.g., condition following myoma enucleation) and/or synechiae or myoma may contribute to insufficient formation of the decidual layer in some circumstances, thus promoting premature placental abruption. Why cocaine increases the incidence of premature placental abruption is unknown. Vascular constriction leading to acute circulatory disturbances in the decidua is discussed here, with subsequent ischemia, necrosis, and hemorrhage (Mbah et al. 2012). Last but not least, this process is also conceivable in cases of excessive elevations of maternal blood pressure or in cases of chronic placental insufficiency with significantly impaired placental perfusion. Thus, pregnant women with hypertensive disease have a fivefold increased risk of severe premature placental abruption compared with normotensive pregnant women. This perfusion disturbance can also occur in nicotine abuse during pregnancy and thus lead to ischemia in the decidual layer, with the consequences of

premature placental abruption (Kaminsky et al. 2007). Interestingly, recent data suggest that administration of vitamins C and E may reduce the rate of premature placental abruption and preterm birth in nicotine abusers in pregnancy in the normal collective (Abramovici et al. 2015). The extent to which thrombophilia (MTHFR gene mutation, hyperhomocysteinemia, factor V Leiden mutation) increases the risk of premature placental abruption is a matter of considerable controversy in the literature (Tikkanen 2011).

Except for the development via trauma, premature placental abruption must ultimately always be interpreted as a process in connection with a chronic change in the placenta, which leads to a change in the vessels in the decidual layer, resulting in ischemia and inflammatory reactions and thus in the development of necroses, infarcts and ultimately hemorrhages. Thrombin plays a key role in the development of premature placental abruption. Thrombin can contribute directly or indirectly to uterine activation of the myometrium (contractions) (Elovitz et al. 2000; Lockwood et al. 2012). In addition, it interferes with the coagulation cascade in the mother and promotes the development of disseminated intravascular coagulopathy (DIC) (Thachil and Toh 2009).

8.4.4 Clinical Signs

There are neither typical clinical signs nor a typical regular course for premature abruption of the placenta. It can be subacute or chronic. In about 80% of cases it is associated with vaginal bleeding. In two thirds of the cases, a hard uterus (wooden uterus) is present. CTG changes are documented in 60% of cases (Hurd et al. 1983). In 20–30% of all cases, the pregnant woman shows no symptoms at all (van de Vondel 2010). A typical symptom is abdominal pain, but also back pain, especially if a posterior placenta is present. In addition, a sudden drop in blood pressure in the pregnant woman can be indicative. Mostly, the clinical picture is diffuse, which leads to a delayed diagnosis and initiation of further steps. This is especially the case if the lower abdominal or back pain is not associated with visible vaginal bleeding (Suzuki 2015). It is not uncommon for premature placental abruption to be diagnosed at the time of delivery (e.g. during cesarean section). However, the signposts for diagnosis are usually lower abdominal pain and vaginal bleeding. The severity of the bleeding does not correlate with the degree of detachment of the placenta and/or fetal vulnerability (Kasai et al. 2015). There is evidence that lower abdominal pain, as a symptom of incipient placental abruption, may underlie circadian rhythms with a higher rate of intrauterine fetal death in the morning hours (Ohhashi et al. 2017). Therefore, such complications should be expected especially during periods of lower staffing density in clinics. In addition to the typical clinical signs, careful attention should be paid to risk factors during diagnosis (◘ Table 8.6) and preterm labor that cannot be explained in any other way (possibly hyperfrequency). For example, if you are caring for a patient with severe preeclampsia, you must expect such a complication at any time and correctly interpret the signs – as opposed to the prodromes typical of preeclampsia.

8.4.5 Instrumental Diagnostics

B-Mode Sonography

Although B-mode ultrasonography usually provides a good view of the placenta, the diagnosis of premature placental abruption is not easy. The expected sonographic image of an echo-poor space between the inner wall of the uterus and the placenta does not show up regularly. The developing hematoma can be hypo-, hyper- or isoechogenic

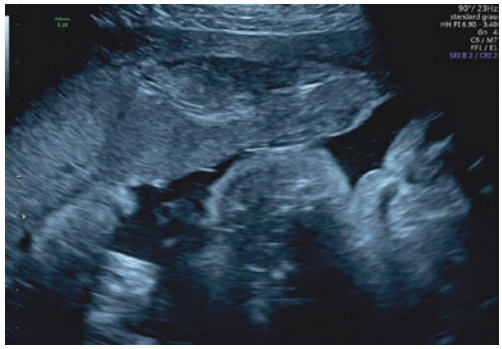


Fig. 8.24 Premature partial placental abruption. The retroplacental hematoma with a diameter of 5 cm was measured in the 32nd week of gestation. The hematoma is located in the periphery of the placenta and can only be blurredly delimited from the placenta

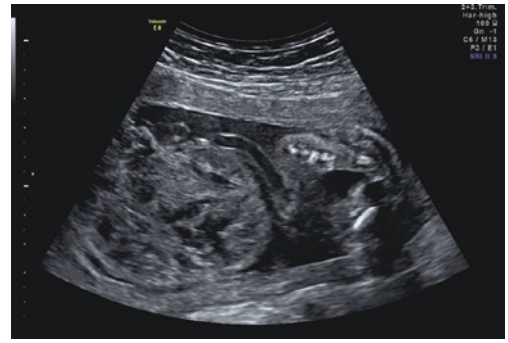


Fig. 8.25 Massive retroplacental hematoma. The placenta cannot be demarcated. The sonographic image appears typically inhomogeneous

compared to the placenta and therefore does not allow a clear diagnosis in all cases. There are partly cystic and partly solid areas, which are only blurredly distinguishable from the placenta. Palpation performed simultaneously with sonography can be helpful. Under certain circumstances, floatation of the blood clots or compressibility of the hematoma is recognizable. The specificity of sonography in diagnosing premature placental abruption is 93% with a sensitivity of 28% (Glantz and Purnell 2002; Shinde et al. 2016). Thus, premature placental abruption can be well diagnosed via B-mode ultrasonography, if visible (Fig. 8.24). However, an inconspicuous sonographic image does not rule it out. Sonographic evidence of premature placental abruption is associated with a worse fetal and maternal outcome (Shinde et al. 2016) (Fig. 8.25).

Doppler Sonography

Not every premature placental abruption leads to an emergency delivery. Sometimes the clinical picture of the patient is not clear, or one dares to wait with the delivery due to an extremely early gestational age – despite the diagnosis of a partial premature placental abruption. It is therefore natural to ask whether Doppler sonography can make a contribution to the diagnostic chain or better determine the optimal time of delivery. However, studies in this direction show that Doppler sonography is not useful in the detection, evaluation and assessment of premature placental abruption. Despite progressive detachment, normal Doppler values are regularly seen in the umbilical cord, which can probably be explained by a loss of placental resistance and the resulting barrier-free flow into the retroplacental space. However, a decreasing resistance in the middle cerebral artery in fetuses after the 32nd week of gestation may be the first indication of fetal asphyxia, and may support the tentative diagnosis of premature placental abruption with the inclusion of all other signs (Morales-Rosello et al. 2017). Therefore, it remains to be stated that Doppler sonography has no value over pathological CTG in the obstetric management of premature placental abruption. However, in ambiguous situations where prolongation of pregnancy is being considered, a middle cerebral artery resistance decrease can be a valuable tool to detect the progressive fetal endangerment situation. However, the physiological decrease in resistance of the middle cerebral artery with increasing gestational age must be taken into account (Gadelha-Costa et al. 2007).

Cardiotocography (CTG)

CTG monitoring is able to indicate an intrapartum change in fetal condition very sensitively. This advantage is contrasted by the low specificity of the method, which is mainly reflected in the large number of false-positive findings. In advanced premature placental abruption, the CTG often shows abnormal heart rate patterns (tachycardia, bradycardia, restricted macrooscillation, decelerations, etc.), which indicate a fetal risk situation but are not pathognomonic. If appropriate, a hyperfrequent labor pattern may be evident. The CTG may remain classified as unremarkable for a long time even in the presence of extensive placental abruption, so that an unremarkable CTG does not rule out premature placental abruption. If the CTG already shows bradycardia when the diagnosis of premature placental abruption is confirmed (e.g. by ultrasound), this is associated in particular with severe fetal acidosis and a poor fetal outcome – irrespective of the gestational age and the sonographic findings (Matsuda et al. 2013; Takano et al. 2013).

Magnetic Resonance Imaging (MRI) and Computed Tomography (CT)

In the clinical diagnosis of acute premature placental abruption, magnetic resonance imaging and computed tomography are not of major importance. Secondarily, however, they can be performed in the context of maternal trauma to exclude further organ damage and can be useful in clinical management. MRI is able to visualize hemorrhage as well as ischemia and inflammatory reactions in and around the placenta (Linduska et al. 2009). Visualization of retroplacental hematoma is more successful than in B-mode ultrasonography (Masselli et al. 2011). In addition to MRI, premature placental abruption in trauma patients can also be diagnosed very well on CT. After trauma, it is not uncommon for a CT to be performed to evaluate for conceivable organ damage to the mother. This can help to better assess the risk for mother and child with regard to premature placental abruption after such an event and to align further clinical management with these findings (Kopelman et al. 2013). A grading system (Traumatic Abruptio Placenta Scale, TAPS) was published in 2014 (Saphier and Kopelman 2014).

8.4.6 Laboratory Diagnostics

Serum Markers in the First and Second Trimester

The serum markers plasma protein A (decreased), alpha-fetoprotein (increased) and inhibin A (decreased) have been discussed for the evaluation of the risk of premature placental abruption in the first and second trimester (Ananth et al. 2017a). However, a valid screening algorithm for clinical practice does not exist (Odibo 2014). Nevertheless, the conspicuousness of serum markers in the context of maternal risk factors (age, previous diseases, etc.) is confirmed in various studies and thus forms the basis for using them in the future for risk evaluation not only in preeclampsia (Odibo 2014; Blumenfeld et al. 2014). However, further studies are still required for this purpose.

Kleihauer-Betke Test

Detection of fetal erythrocytes in maternal blood cannot be used to diagnose premature placental abruption, as a positive Kleihauer-Betke test does not prove premature abruption and a negative test does not rule it out (Dhanraj and Lambers 2004). A recent paper reported a sensitivity of 4.4% for the Kleihauer-Betke test in cases of premature placental abruption (Atkinson et al. 2015a).

Blood Count/Coagulation Diagnostics

Maternal blood loss in premature placental abruption is reflected in the laboratory as a drop in the Hb value in the complete blood count. It is not uncommon for the blood loss to be accompanied by a coagulation disorder, which is best visible in the laboratory on the basis of fibrin cleavage products (fibrinogen level, thrombin time) and thrombocytopenia (<80,000/ml). Thus, it is possible to predict a less favorable maternal and fetal outcome of premature placental abruption by prenatal determination of the fibrinogen level (<155 mg/dl) (Wang et al. 2016). A marked increase in thrombin indicates progressive DIC and fibrinolysis. Laboratory checks should be done 4-hourly. In the event of rapid clinical deterioration, laboratory checks are indicated on a more goal-directed, close-meshed basis.

8.4.7 Clinical Care/Management

The clinical care or management of a patient affected by premature placental abruption depends mainly on three factors:

- Gestational age,
- Fetal condition (fetal endangerment, intrauterine fetal death),
- Maternal state (shock symptomatology).

Obstetric management is therefore geared to the maternal and fetal risk situation, which unfortunately often only becomes fully apparent retrospectively (Table 8.7).

The lower the gestational age of the child, the more one will strive – in view of the fetal risks of premature birth (see following overview) – for a wait-and-see management with close fetomaternal controls and try to prepare the fetus for a premature birth (lung maturation induction, neuroprotection, etc.). The more advanced the child's maturity in the womb, the more emphatically delivery is sought in order to keep the spectrum of possible complications (see following overviews) low.

Table 8.7 Clinical classification of premature placental abruption according to severity. (Modified according to Page et al. 1954)

Grade	Characteristics
0	Asymptomatic patient Diagnosis sonographically or after delivery Fetus not affected
I	Little bleeding inwards or outwards Low pressure painfulness of the uterus with slight increase in tone No impairment of the maternal circulatory situation Fetal impairment rare but possible
II	Moderate internal or external hemorrhage with compensated maternal circulatory situation Uterine contractions Signs of fetal endangerment
III	Severe bleeding internally or externally Extremely pressure-painful uterus with abdominal defensive tension Maternal shock, in 30% with coagulopathy Child severely impaired or deceased

Page et al. (1954), Tikkanen (2010), Kainer et al. (2014)

Childhood Risks of Premature Placental Abruption (Downes et al. 2017)

- Increased risk of neonatal resuscitation (relative risk [RR] 1.5; 99% confidence interval [CI]: 1.5–1.6).
- Apnea (RR 5.8; 99% CI: 5.1–6.5).
- Asphyxia (RR 8.5; 99% CI: 5.7–11.3).
- Respiratory distress syndrome (RR 6.5; 99% CI: 5.9–7.1).
- Necessary care in a neonatal intensive care unit (RR 3.4; 99% CI: 3.2–3.6).

- Prolonged intensive care (RR 2.0; 99% CI: 1.9–2.2).
- Risk of stillbirth (RR 6.3; 99% CI: 4.7–7.9).
- Increased neonatal mortality (RR 7.6; 99% CI: 5.2–10.1).

Nevertheless, a significant fetal or maternal risk (see following overview) forces the delivery, regardless of gestational age. In this context, perinatal mortality is significantly higher in infants born before 30 weeks gestation compared with those born at higher gestational ages (42% vs. 15%) (Atkinson et al. 2015b). However, effects of premature placental abruption on children's long-term neurocognitive development do not differ compared to other preterm infants, so corresponding abnormalities must be attributed to gestational age at delivery (preterm birth per se) (Ananth et al. 2017b).

Maternal Risks Associated with Premature Placental Abruption

(Tikkanen 2011; Yang and Sun 2017; DeRoo et al. 2016; Ananth et al. 2016; Ananth et al. 2017a, b, c).

- Complications in the course of an emergency caesarean section
- Hemorrhage (coagulation disorder, compulsory transfusion, shock, multiple organ failure)
- Sevenfold increased mortality risk
- Increased risk of developing cardiovascular disease later in life (1.8-fold increase in mortality)

If there is a high danger to the mother and/or child, delivery should be induced immediately. This is usually done by cesarean section, unless a vaginal birth can take place immediately. The latter is conceivable, for example, in the case of a placental detachment immediately before partus or in the context of a twin delivery. In addition, vaginal delivery may be considered in cases of stable maternal clinic and intrauterine fetal death (IUFD) (Imanaka et al. 2014; Inoue et al. 2017). In the event of rapid clinical deterioration of the pregnant woman, rapid delivery by cesarean section should also be sought (Okafor and Ugwu 2015). Delivery complicated by premature placental abruption must be expected to have significant bleeding complications. This particularly highlights the importance of the logistics of a modern interdisciplinary perinatal center (midwives, obstetricians, neonatologists, pediatric nurses, anesthetists, operating theatre staff, etc.) in order to achieve an optimal outcome in maternal and fetal care.

After delivery, the placenta should regularly be sent for histopathological examination. Retroplacental hemorrhages can be detected in 77.1% of cases. In most cases, the changes are non-specific or indicate ischemic or inflammatory processes. It should be noted that the changes in the placenta are time-dependent, and acute resolution therefore shows few histopathological changes (Tikkanen 2010). Thus, the sensitivity for histopathological confirmation of premature placental abruption is 30.2% with a specificity of 100% (Elsasser et al. 2010). In any case, the findings – even in the case of inconspicuous histopathology – can be helpful in shaping the counselling for a further pregnancy. There are no effective preventive measures. However, close-meshed prenatal care and risk evaluation can help to favorably choose the time of delivery in order to anticipate a possible placental abruption.

Often, after the traumatic experience of a placental abruption, parents come to the consultation with the question of the risk of recurrence in a new pregnancy. This amounts to 5–17%, and after two abortions the risk

rises to 25%. In this case, consistent monitoring of the subsequent pregnancy, taking into account the risk parameters that can be influenced or controlled, can help the parents to end the pregnancy well accompanied. At the same time, the obstetrician will find it easier to assess a renewed placental abruption if he/she is aware of the risk.

8.4.8 Conclusion

Premature placental abruption cannot always be diagnosed in routine clinical practice from the outset. Therefore, knowledge of the risk factors (Table 8.6) for premature placental abruption in the diagnostic chain (anamnesis, clinic, instrumental diagnostics, etc.) is of great importance. Only in this way can the whole obstetric picture be recorded promptly so that appropriate management can be designed according to the degree of severity (Table 8.7). This makes a significant contribution to reducing infant and maternal morbidity and mortality. In the event of significant maternal and/or fetal danger, rapid, decisive interdisciplinary action – if possible in a perinatal center – is required to keep maternal and fetal morbidity and mortality as low as possible (Boisram et al. 2014).

8.5 Primary and Secondary Tumors of the Umbilical Cord and Placenta

Lars-Christian Horn

Tumors of the umbilical cord and placenta are extremely rare. Benign tumors predominate at both localizations. The neoplasms are usually secondary tumors.

8.5.1 Tumors of the Umbilical Cord

Tumors of the umbilical cord are primary tumors.

The majority of **hemangiomas** of the umbilical cord are incidental findings. To date, slightly more than 35 cases have been published (Papadopoulos et al. 2009). In most cases, the microscopic tumors are discovered by chance during examination of the umbilical cord. Larger tumors may come to light by Doppler sonography (Moshiri et al. 2014). There is a slight female predominance, and the tumors occur predominantly in the portion of the umbilical cord near the fetus. Pathogenetically, there is an association with the umbilical arteries, and an origin from angioblasts of Wharton's jelly seems possible.

Angiomyxomas of the umbilical cord (Cheng et al. 2006; Tennstedt et al. 1998) are probably not a distinct entity but hemangiomas with proliferation and regressive changes in Wharton's jelly (Emmrich and Horn 1992).

Hemangiomas may rarely be associated with fetal malformations, hydramnion, prematurity, or elevated maternal AFP levels (Papadopoulos et al. 2009).

Teratomas of the umbilical cord are extremely rare (Satgé et al. 2001) and usually benign. Some of the cases described are probably fetus acardius amorphus (Kreczy et al. 1994). The differential diagnosis is discussed in section "Teratomas of the placenta".

True (dysontogenetic) **umbilical cord cysts** are very rare and must always be differentiated from an omphalocele and cystically transformed portions of the allantoic duct (Baergen 2005; Zangen et al. 2010).

8.5.2 Tumors of the Placenta

According to their origin, tumors of the placenta can be divided into primary and secondary tumors (Fig. 8.26). A few tumor-like lesions may mimic clinically true tumors.

Primary Non-trophoblastic Tumors of the Placenta

Teratomas

Teratomas are extremely rare (Prashanth et al. 2012) and can occur in the umbilical cord as well as the chorionic plate. In analogy to teratomas of other localization, they consist of derivatives of all three germ layers and arise from aberrantly migrated embryonic stem cells originating from the posterior wall of the yolk sac (Shimojo et al. 1996; Ahmed et al. 2004).

The most important differential diagnosis is the fetus acardius amorphus (Tzelepi et al. 2007). In contrast to teratomas, this shows its own umbilical cord and has an axial orientation, so that in most cases either an axial skeleton, ribs, pelvic structures or a skull base are detectable (Vogel 1996; Baergen 2005).

Malignant teratomas of the placenta have not been described so far.

Chorangiomas, Fibromas and Chorangiocarcinoma

Chorangiomas are defined as benign tumors originating from the vessels of placental villi with an insufficiently clarified pathogenesis (Benirschke 1999; Baergen 2005). It is likely that growth is triggered by angiogenic factors. Although chorangioma is the most common placental tumor, chorangiomas are very rare. Their incidence is reported to be 1:9000 to 1:50,000 placentas (Benirschke 1999; Baergen 2005). With systematic workup of placentas, it increases to 1:100 to 1:250 (Baergen 2005; Vogel 1996), which

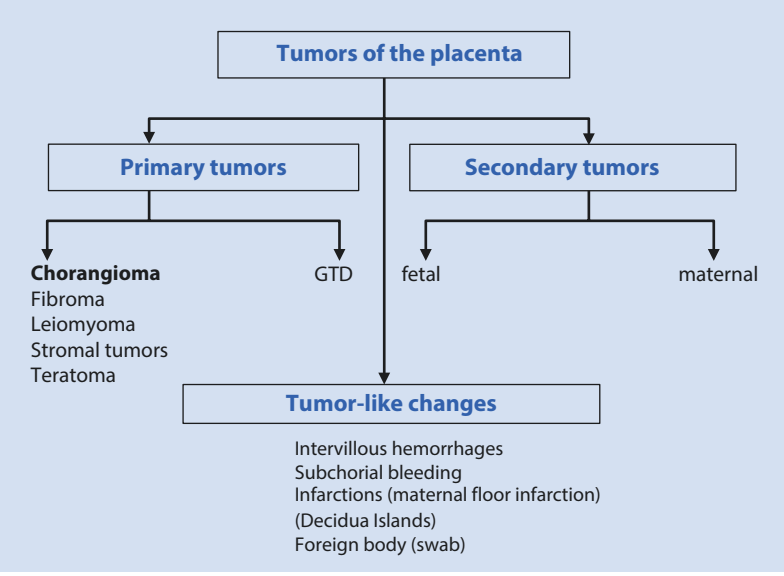


Fig. 8.26 Classification of tumors of the placenta

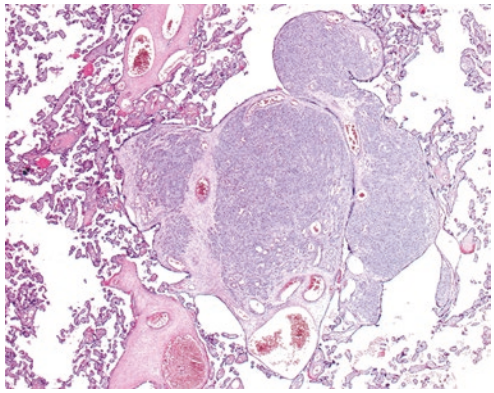
corresponds to an incidence of 0.41% (Becker 1981).

More than 75% of cases are microscopic incidental findings, so-called microchorangiomas (Becker 1981; Vogel 1996) (◘ Fig. 8.27). Unlike sonographically and macroscopically visible chorangiomas (◘ Fig. 8.28), microchorangiomas are not expected to be clinically symptomatic. Microchorangiomas are located in the placental parenchyma without predilection for localization. Macrochorangiomas are subchorial in location (◘ Fig. 8.29) and rarely larger than 5 cm (Fan and Skupski 2014).

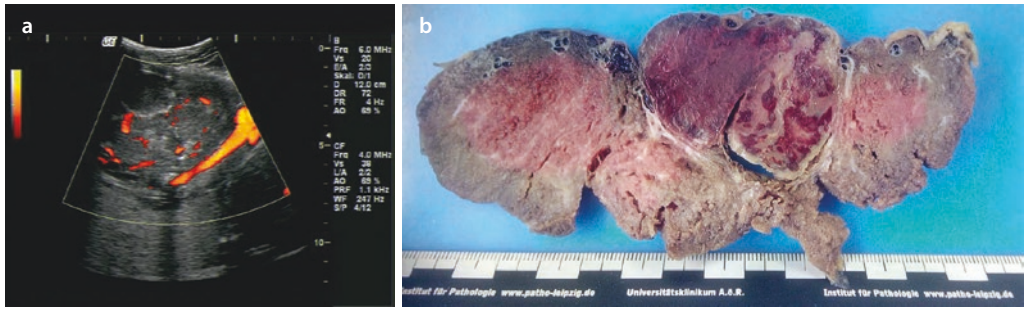
According to their vascular morphology, endotheliomatous, capillary, fibromatous and cavernous forms of chorangioma can be distinguished (Becker 1981; Baergen 2005), although this has no clinical significance. The majority of macrochorangiomas also have a mixed morphology (◘ Fig. 8.29).

Regressive changes such as hemorrhages, thromboses, infarcts and necroses as well as calcifications are known (Vogel 1996; Baergen 2005), which can be misinterpreted sonographically. Due to extensive connective tissue changes, fibrosis may be prominent. Previously, these tumors were referred to as fibromas (Becker 1981); in doubtful cases, vessels can be detected immunohistochemically using CD 31, CD 34, or anti-factor VIII. Within the chorangiomas, smooth muscle fibers may proliferate, so that a combination of chorangioma and leiomyoma-like changes may be present (Miliaras et al. 2011).

Chorangiomas are superficially covered by trophoblastic epithelium, which may occasionally show hyperplasia (◘ Fig. 8.29). This focal hyperplasia should be distinguished from intramolar choriocarcinoma as a special form of gestational trophoblastic disease (see below) and the so-called **chorangiocarcinoma** (Ariel et al. 2009; Faes et al. 2012). Chorangiocarcinoma is defined as an entity that presents with the appearance of a chorangioma but superficially shows severe trophoblastic cell hyperplasia,



◘ **Fig. 8.27** Histological overview of a so-called microchorangioma of the placenta



◘ **Fig. 8.28** **a, b** Macrochorangioma of the placenta. **a** Doppler sonographic image of a (macro)chorangioma (courtesy of Dr. S. Schrey-Petersen, University Hospital Leipzig AöR). **b** Macroscopic image of a chorangioma located under the chorionic plate

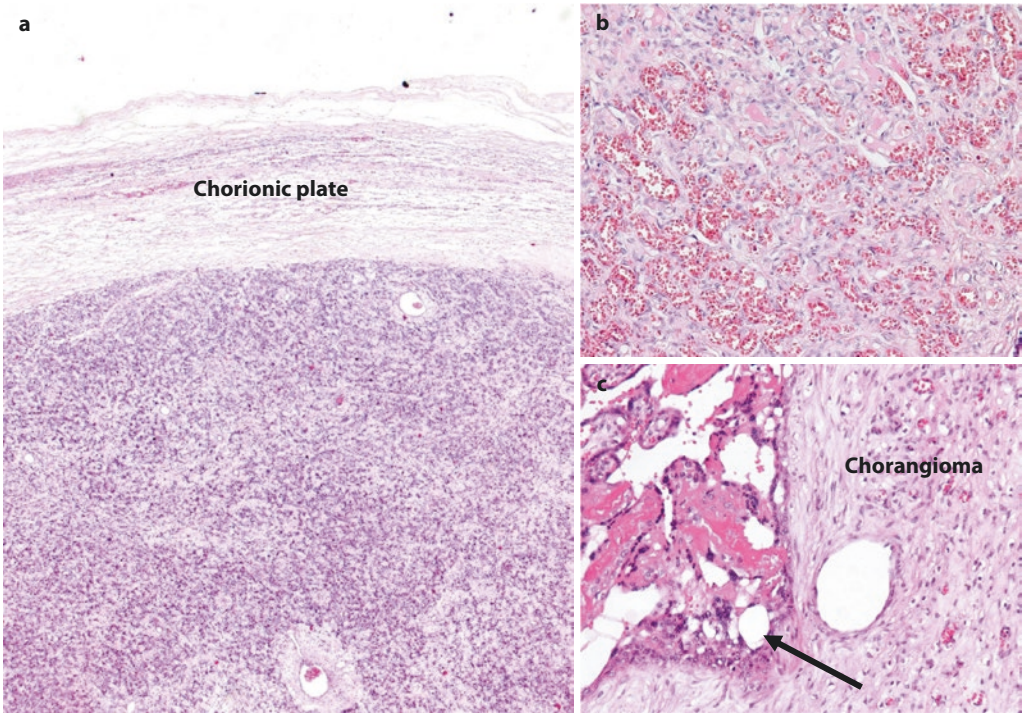


Fig. 8.29 **a–c** Histology of chorangioma. **a** Chorangioma localized under the chorionic plate. **b** Gross enlargement of a chorangioma with capillary and endotheliomatous morphology (see text). **c** Reactive trophoblast cell hyperplasia at the surface of a chorangioma (*arrow*, see text)

occasionally with atypia (Ariel et al. 2009; Faes et al. 2012).

Occasionally, chorangiomas may show a cell-rich stroma with atypia (Vellone et al. 2015), but this has no clinical relevance.

Morphologically, chorangiomas are to be distinguished (Ogino and Redline 2000; Baergen 2005) from:

- **Chorangiomatosis**, which is defined as a proliferation of intravillous vessels in the stem and/or intermediate villi (Fig. 8.30), mostly without involvement of the resorptive (terminal) villi (Baergen 2005, Vogel 1996). The vessels are surrounded by a loose stroma. Chorangiomatosis is usually an incidental finding, but may be visible macroscopically as a multinodular change in the placental parenchyma and may occur segmentally as well as diffusely. Morphologically there are overlaps to chorangiomas. Similar to chorangiomas,

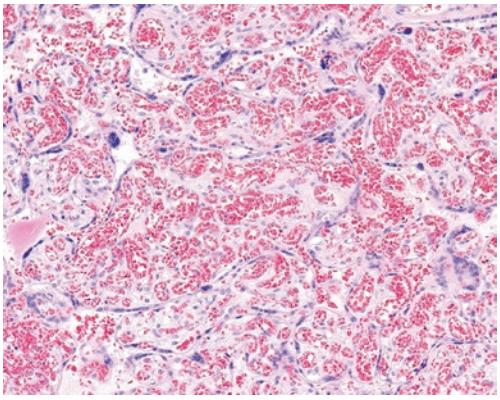


Fig. 8.30 Chorangiomatosis of the placenta

chorangiomatosis may be associated with prematurity, preeclampsia, growth restriction, placentomegaly and malformations (Ogino and Redline 2000).

- **Chorangiosis** is characterized as a diffuse proliferation of small vessels in small intermediate and resorptive terminal villi (Gupta et al. 2006) (Fig. 8.30). Baergen (2005) defined chorangiosis as a purely histopathological diagnosis with evidence of >10 vessels in each of the above classes of villi in at least ten villi in ten microscopic fields of view at 100× magnification in at least three different locations in the placenta. The vessels are surrounded by a basement membrane. Chorangiosis may be associated with preeclampsia (Gupta et al. 2006).

The clinical significance of chorangiomas correlates closely with their size (Benirschke 1999; Baergen 2005; Vogel 1996; Fan and Skupski 2014). Fetal and maternal complications associated with chorangiomas are summarized in the following review and are a consequence of altered hemodynamics, tumor size itself, or increased fluid transudation. In very rare cases, a combination of chorangiomas and fetal vascular tumors has been described (Selmin et al. 2014).

Synopsis of Placental Chorangiomas and Their Differential Diagnoses

Chorangiomas, chorangiosis and chorangiomatosis are related vascular proliferations of the placenta and defined as:

- Chorangioma: Benign focal, nodular vascular proliferation originating from stem villus vessels
- Chorangiomatosis: Multifocal or segmental vascular proliferation in stem or (larger) intermediate villi
- Chorangiosis: (Diffuse) vascular proliferation in (small) intermediate and terminal villi

In differential diagnosis, the following can be delineated:

- Chorangiocarcinoma: Chorangioma with strong trophoblast proliferation showing morphologic features of choriocarcinoma.
- Incidence 1:9000 to 1:50,000 placentas (with systematic workup up to 1:100–1:250)
- 75% are only histologically detectable microchorangiomas; 25% are macroscopically/sonographically visible
- Large chorangiomas are characteristically located subchorial
- Regressive changes are: bleeding, thrombosis, necrosis, calcification
- Histopathological subtyping into endotheliomatous, capillary, cavernous as well as extensive fibrosis and a cell-rich, occasionally atypical stroma have no clinical relevance
- Covering trophoblastic epithelium may be (reactively) proliferating, without clinical significance → chorangiocarcinoma (see above) must be excluded
- Clinical significance/complications correlates with size of chorangioma (review "Fetal and maternal disorders associated with chorangiomas of the placenta")

(Baergen 2005; Vogel 1996; Benirschke 1999; Isaacs 2008; Faes et al. 2012; Fan and Skupski 2014).

Fetal and Maternal Disorders Associated with Chorangiomas of the Placenta

1. **Maternal Complications**
   - Polyhydramnion
   - Prematurity
   - Preeclampsia

2. **Fetal Complications**
   - Thrombocytopenia, hemolytic anemia[a]
   - Hemorrhage from chorangioma with fetal hemorrhagic anemia or hemorrhagic shock[a]/death

- Premature abruption of the placenta[a]
- Growth restriction in chronic placental insufficiency
- Cardiomegaly due to intratumoral shunt formation[a]
- Hydrops fetalis[a]

[a]Association with increased perinatal mortality

(Vogel 1996; Baergen 2005; Amer and Heller 2010; Ogino and Redline 2000; Fan and Skupski 2014; Selmin et al. 2014; Abiramalatha et al. 2016; Al Wattar et al. 2014; Wu and Hu 2016).

Leiomyomas, Endometrial Stromal Tumors, Deciduomas, Hepatocellular Adenomas

Intraplacental leiomyomas have been described (Tapia et al. 1985). They are mostly located near the basal plate and are covered by decidua. Because of the lack of connection to the uterus, they are interpreted as primary leiomyomas of the placenta. However, it must be remembered that there is no intrinsic smooth muscle tissue within the placenta that can serve as a point of origin. Molecular analyses in individual cases could prove that the intraplacental leiomyoma originates from the mother (Ernst et al. 2001; Tarim et al. 2003). Therefore, it is currently assumed that intraplacental leiomyomas are not primary tumors but so-called parasitic (submucous) leiomyomas of the uterus, which no longer have a (detectable) connection to the myometrium and were incorporated into the placenta during pregnancy.

The same is true for **endometrial stromal tumors**, which may rarely occur within the placenta (Katsanis et al. 1998; Karpf et al. 2007).

The so-called **deciduomas** described in the older literature (Davies 1948) are primarily endometrial stromal tumors with high-grade decidualization, as known in lesions arising during pregnancy (Katsanis et al. 1998; Chew and Oliva 2010).

Hepatocellular adenomas can occur in the placenta (Khalifa et al. 1998). Pathogenetically, they derive from scattered yolk sac cells and show a benign course. Rarely, they may be associated with a chorangioma (DeNapoli 2015).

Intraplacental Heterotopias

Heterotopic tissue in the form of adrenal, liver and brain tissue can be seen in the placenta (Baergen et al. 1997; Guschmann et al. 2000). Pathogenetically, it is assumed that this is primarily a vascular carry-over via fetal vessels. A monodermal teratoma or abnormal mesodermal differentiation is also discussed. The detection of heterotopic tissue has no clinical relevance.

Secondary Tumors of the Placenta

Maternal Tumors

The incidence of malignant tumors is 1:1000 pregnancies (Triunfo and Scambia 2014). Malignant melanoma is the most common neoplasm (Driscoll et al. 2016).

Metastases in the placenta are very rare, with fewer than 100 published cases (Pavlidis and Pentheroudakis 2008). They are usually advanced tumors of the mother (Jeong et al. 2014). The most common tumor of origin is malignant melanoma, followed by breast carcinoma, gastrointestinal tumors, tumors of the cervix and lung; other tumors are extremely rare (Reif et al. 2014).

Transplacental metastasis to the fetus is extremely rare with just under 20 cases published to date (Dildy et al. 1989; Reif et al. 2014). It is assumed that the villous trophoblast represents a natural barrier with regard to invasion from the intervillous space (maternal vascular compartment) into the chorionic villi and their vessels (fetal vascular compartment). Even with intravillous infiltration of maternal tumors into the chorionic villi, proven metastases in the child are rare.

Clinically, every placenta of a pregnant woman with a known malignant tumor should be examined histopathologically and if there is evidence of placental involvement by the maternal tumor in the child, metastasis should be excluded.

Fetal Tumors

Fetal tumors with involvement of the placenta are extremely rare. In the majority of these cases, this is limited to the fetal compartment (chorionic villi) or the intervillous space, and in only four well-documented cases has metastasis to the maternal organism been identified (Reif et al. 2014).

The three most common fetal tumors with placental involvement are neuroblastoma, hepatoblastoma, and congenital leukemias (Roberts and Oliva 2006; Kume et al. 2014; Wolf et al. 2017) (Fig. 8.31). A coincidence of hydrops fetalis and placentomegaly is very commonly seen, with fetal heart failure as the cause of death (Kume et al. 2014; Wolf et al. 2017). When a congenital malignant tumor is detected, an extensive placental examination should be performed and the (often very immature) tumor cells detectable in the vessels of the chorionic villi should not be confused with erythoblastosis, so that immunohistochemical clarification should always be performed.

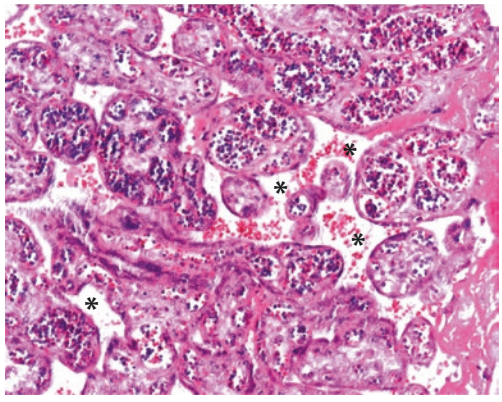


Fig. 8.31 Detection of cells of congenital fetal acute lymphoblastic leukemia (ALL) in vessels of the chorionic villi (fetal vascular compartment) but not in the intervillous space. * Maternal vascular compartment

Primary Trophoblastic Tumors of the Placenta

Intraplacental chorionic carcinomas are extremely rare, accounting for <1% of all gestational trophoblastic tumors (Sebire et al. 2005; Kanehira et al. 2013). In the majority of cases, these have a good prognosis, without maternal metastases, which have however been described (mainly in the lungs) (Jacques and Qureshi 2011; Landau et al. 2006).

Mostly, they are histological incidental findings, very rarely intraplacental choriocarcinomas may be associated with fetomaternal hemorrhages, retroplacental hemorrhages, hydrops fetalis and intrauterine growth restriction (Liu and Guo 2006).

To date, only three cases with fetal and maternal metastases have been reported (Jacques and Qureshi 2011).

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Placental Imaging

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9.1 Sonographic Assessment of the Placenta in the Second and Third Trimester and Ultrasound/MRI Morphology of the Placenta

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9.1.1 Introduction

The examination of the placenta usually receives less attention than that of the fetus, often not being mentioned as part of a routine ultrasound examination in an unremarkable pregnancy. However, the placenta is a key organ in the development and status of the fetus and its assessment should therefore be an obligatory part of any ultrasound examination in pregnancy.

Methodological ultrasound examination of the placenta includes: determination of localization, umbilical cord insertion and implantation, placental thickness, size and shape, as well as structure and maturity, and the search for cystic lesions, tumors and abnormal echogenicity. Failure to detect these placental pathologies may increase the risk for adverse outcome for mother and fetus. Proper diagnosis allows interventions that can improve perinatal and maternal outcome. On the other hand, inconclusive findings of unclear significance in routine ultrasound can lead to uncertainty and anxiety. Therefore, it is important to be familiar with the normal placental anatomy and its variants.

Examination and imaging of the placenta is an important component of obstetric monitoring. The placenta should be sonographically examined and documented in all pregnant women (Merz et al. 2012).

Criteria for Sonographic Placental Assessment

The differentiated examination of the placenta includes the assessment of

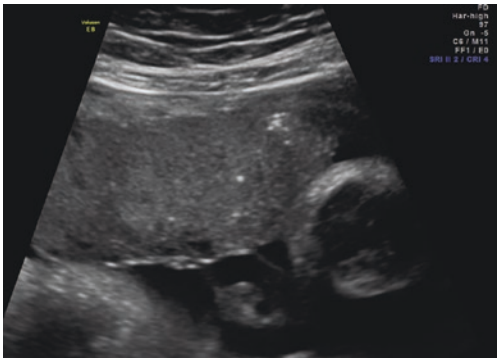
- Shape
- Size and thickness
- Echo-structure and maturity
- Echogenicity
- Localization
- Tumors
- Implantation disorders
- Umbilical cord

The standard examination method for imaging the placenta is still sonographic assessment using 2D/3D procedures and Doppler sonography. At higher weeks of gestation and under limited examination conditions (obesity, oligo-/anhydramnios unfavorable fetal position or posterior placenta), sonographic assessment of the placenta may be limited and magnetic resonance imaging (MRI) may be helpful, especially for specific questions (Dekan and Linduska 2011). Advantages of MRI are the large field of view (FoV), soft tissue differentiation, and multiplanarity. In addition, some pathologies such as placental implantation disorders and, in some cases, infarctions and hemorrhages can be better visualized using MRI (Dekan and Linduska 2011; Jha et al. 2016).

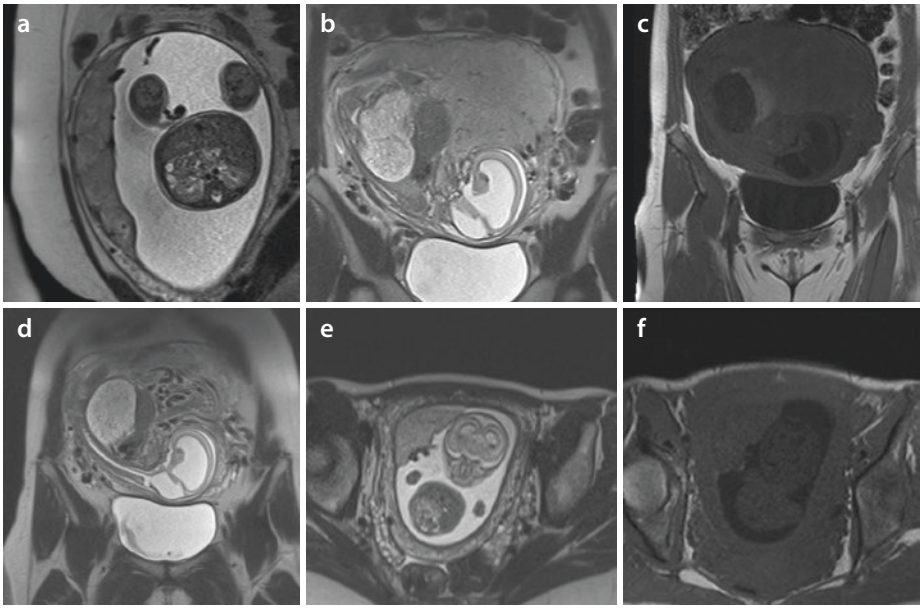
The normal placenta appears ultrasonographically as a homogeneous intermediate echogenic structure adjacent to the internal myometrium, from which it is separated by a hypoechogenic border zone (Fig. 9.1).

Depending on the MRI sequence, the placental MRI signals vary. In the T2-weighted multiplanar single-shot echoplanar turbo/fast spin echo sequences acquired in three spatial planes (axial, sagittal, coronal), which are used as a standard technique for fetal

MRI examinations (e. g. HASTE, "half Fourier acquisition single shot turbo spin echo"; SSFSE, "single shot fast spin echo"; UFSE, "ultra fast spin echo" etc.; named according to manufacturer), the placenta appears as homogeneous tissue, isointense to the myometrium in early pregnancy. A thin hypointense line may represent the placenta-myometrium boundary. As pregnancy progresses, the placental signal becomes hypointense compared to the myometrium. On T1-weighted sequence ("T1w spoiled gradient echo", with frequency-selective fat saturation or opposed phase imaging), which should be acquired in at least one spatial plane, the placenta presents hypointense to somewhat hyperintense depending on physiological changes and vascular supply (Jha et al. 2016). The calcifications seen as the placenta ages are poorly differentiated on MRI; if large enough, they can be delineated from the surrounding area without signal. Susceptibility-weighted sequences (SWI) or diffusion-weighted sequences (DWI) with quantitative assessment of diffusivity (ADC determination, "apparent diffusion coefficient") can be helpful in the dedicated assessment of the placenta (Bonel et al. 2010). The use of MRI contrast agents containing gadolinium is not indicated (■ Fig. 9.2).



■ **Fig. 9.1** Normal findings in ultrasound examination



■ **Fig. 9.2** **a–f** Normal findings in MRI. **a** Anterior wall placenta in 30th week of gestation, delineate delicate hypointense (dark) borderline between myometrium and placenta; polyhydramnios (T2w Haste). **b–d**: Fundal placenta in the 15th week of gestation, anhydramnios and pulmonary hypoplasia in polycystic kidneys, hydrocephalus, cervical cele (b: T2w Haste, c: T1fl2d, d: T2w Haste). (**e** and **f**): 19th week of gestation with syndromal disease. Spina bifida (e: T2w, f: T1fl2d)

This chapter is dedicated to the normal and the abnormal placenta in the second and third trimester with special attention to the sonographic appearance, the valuable use of MRI and the potential management.

9.1.2 Localization

Determination of placental localization is the most commonly performed examination of the placenta and is important for risk assessment.

While the actual placental location (anterior, posterior, fundal, lateral) is only of decisive importance in cases of low-lying, the distance of the placenta from the internal cervical os is important for obstetric management and should be assessed by vaginal sonography from the second trimester onwards (Farine et al. 1990).

Lateral placental localization may possibly lead to differences in resistance indices of the uterine arteries, with the ipsilateral uterine artery having lower indices compared to the contralateral side (Ito et al. 1990).

Low-Lying Placenta

The placenta is normally located on the anterior or posterior wall of the uterus and extends to the lateral walls. The caudal edge should be at least 2 cm away from the internal cervical os; a distance of ≤2 cm, without overlapping the cervix, is called a low-lying placenta (Fig. 9.3) (Reddy et al. 2014).

Due to the stretching and growth of the lower uterine segment in the course of pregnancy, a relative "positional change" of the placenta in cranial direction can occur up to about 32 weeks gestation, this is called "cephalad placental migration." These changes can have important consequences for clinical management, especially in the case of a low-lying placenta (Hung et al. 1999).

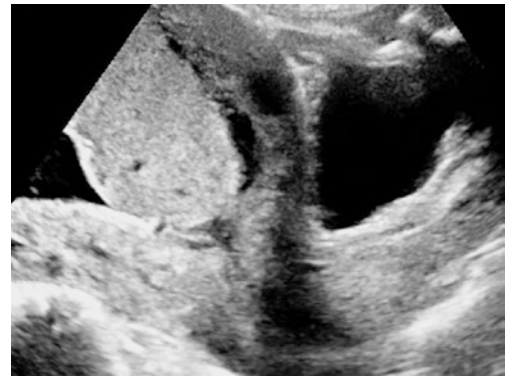


Fig. 9.3 Low-lying placenta

The frequency of a low-lying placenta or placenta praevia depends on the gestational age: Between the tenth and 20th week of gestation, up to 6% of pregnant women present with placenta praevia (Oyelese and Smulian 2006); in the 18th–22nd week of gestation, 2–4% of all placentas reach or overlap the internal cervical os (Oppenheimer et al. 2007), and a low-lying placenta is present in 8.4% (Blouin and Rioux 2012). In 95% of placentas classified as low-lying or overlapping in the second trimester, the diagnosis was revised in the third trimester. This means that normal placental localization in the first trimester (here the anterior and posterior walls of the lower uterine segment are still adjacent) should not be misinterpreted as placenta praevia.

If placenta praevia is "detected" in the first trimester, the diagnosis should be communicated cautiously, and follow-up examinations should be performed in the second and third trimesters (Fuchs et al. 2008). At term delivery, approximately 0.5% of placentas reach or overlap the internal cervical os (Rosenberg et al. 2011).

However, the degree of overlap correlates with the risk of a persistent finding: if the placenta overlaps the internal cervical os <1.5 cm during the examination at 20–23 weeks gestation, there is placenta praevia at term in only

20%. If the overlap is >2.5 cm, the finding persists in 40% (Becker et al. 2001).

A measurement of the distance between the placenta and the inner cervical os in the second trimester should be repeated again around the 34th week of gestation. Only then should a decision be made regarding the mode of delivery because of the placental location.

A distance of the placenta from the internal cervical os ≥2 cm is not associated with an increased risk of bleeding and is considered "safe" for vaginal delivery (Oppenheimer et al. 1991, 2001).

With a low-lying placenta, the C-section rate is 31% and the likehood of obstetric bleeding is 3%. If a low-lying placenta is associated with a marginal sinus, intrapartal hemorrhage is more common. A marginal sinus is described as an area filled with maternal blood and should not be misinterpreted as vasa praevia (fetal vessels in close proximity to the internal cervical os). If the placenta is low lying, vasa praevia can be a subsequent complication with potentially serious consequences.

Placenta Praevia

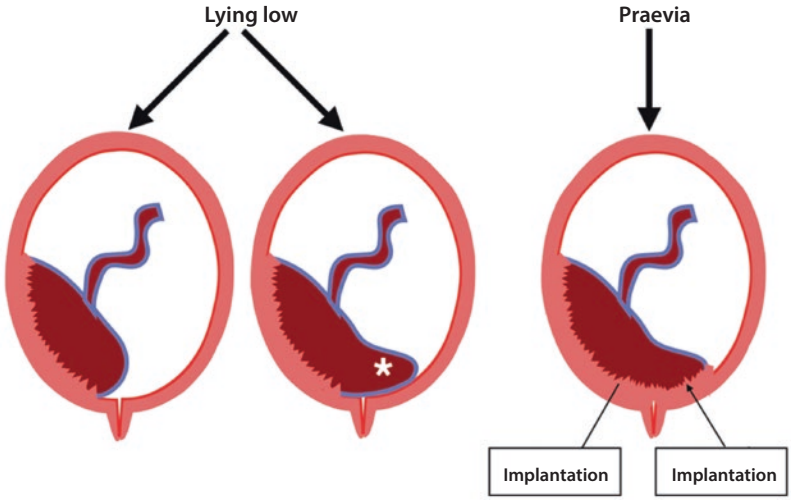
In placenta praevia, the placenta completely covers the internal cervical os (Reddy et al. 2014).

According to Reddy et al. (2014), the classification depends on the relationship to the internal cervical os (see following overview and ◘ Fig. 9.4).

Classification of Placental Localization (According to Reddy et al. 2014)

- Low-lying placenta: distance to the internal cervical os <2 cm
- Placenta praevia: placenta covers the internal cervical os

Due to the almost universal use of ultrasound, a diagnosis of placenta praevia is also made in asymptomatic patients during second trimester screening. Risk factors for placenta praevia are previous uterine surgeries, mainly previous C-sections. If placenta praevia is diagnosed, an implantation disorder (placenta accreta spectrum, PAS) must be considered and excluded (Yang et al. 2007).



◘ **Fig. 9.4** Scheme of placental localization

Diagnosis and thorough evaluation of a Low-lying placenta or placenta praevia by sonography and MRI, if necessary, is crucial for clinical management (Schlembach et al. 2016) because of the increased risk of the placental accreta spectrum, umbilical cord attachment pathology and vasa praevia, and respective pregnancy complications (Hung et al. 1999; Gemer and Segal 1994).

In the case of a low-lying placenta or placenta praevia, the documentation/notification of the placental localization (anterior, posterior) is important for the surgical procedure, since an anterior wall placenta can lead to complications due to the transplacental delivery of the child (umbilical cord hemorrhage, difficult child development) (Fuchs et al. 2008).

For the correct diagnosis of placenta praevia, both the lower placental margin and the internal cervical os must be accurately identified, if necessary also by means of transvaginal ultrasound. This is all the more important as, in the case of placenta praevia, primary C-section must be performed between the 36th–37th week of gestation before the onset of labor.

However, ultrasound is still often inaccurate and incorrect in the diagnosis of placenta praevia. Placenta praevia can be mistaken for a low-lying placenta, which overlaps the internal cervical os but which has not implanted into the opposite segment.

Color Doppler can be used to distinguish between truly implanted and non-implanted placental tissue.

Another source of error is the distinction between the lower uterine segment and the cervix. The uterine isthmus is usually closed during early pregnancy and only opens at higher gestational age. Due to the improved resolution of newer ultrasound machines, recent studies have been able to address the distinction between these two structures and assess the length of the cervix and isthmus (Greco et al. 2011; Souka et al. 2011; Hasegawa et al. 2017). A reliable diagnosis of placenta praevia is made using transvaginal ultrasound between the 20th and 24th week of gestation, after opening of the lower uterine segment and by accurately distinguishing between the cervix and isthmus (Figs. 9.5, 9.6, and 9.7) (Hasegawa et al. 2017).

Other causes of misinterpretation are a full bladder pressing on the lower uterine segment and segmental uterine contractions.

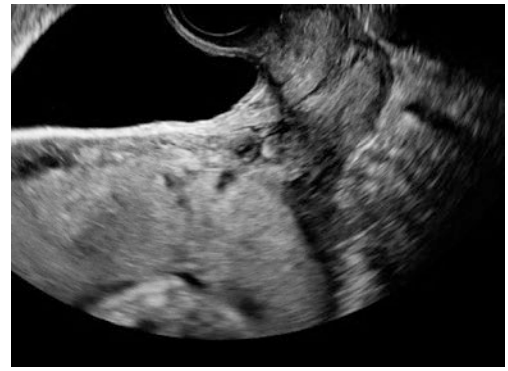


Fig. 9.5 Placenta praevia

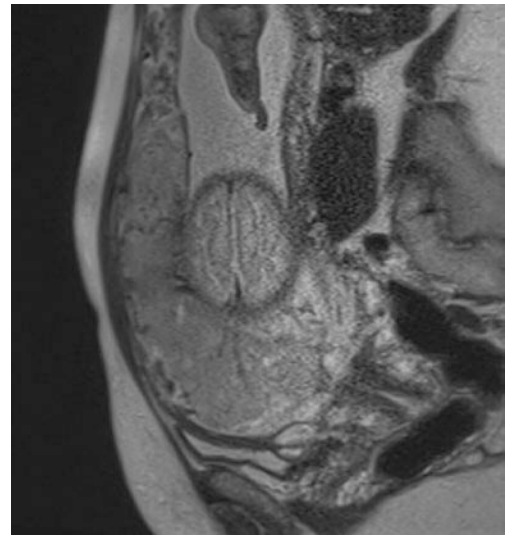


Fig. 9.6 Placenta praevia. T2-weighted HASTE sagittal—the placenta covers the cervix incompletely in the anterior wall region (32nd week)

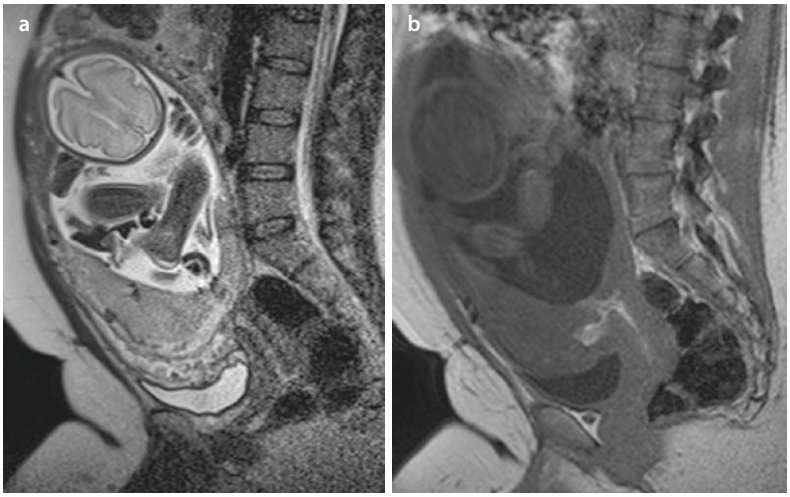


Fig. 9.7 **a**, **b** Placenta praevia. T2-weighted sequence sagittal **a** with displacement of the placenta in front of the cervix. The basal decidual membrane to the myometrium appears intact. T1-weighted sequence **b** with hyperintense mucus plug and irregular placental hemorrhage

In all pregnant women, the placental location should be examined sonographically and, in case of a low-lying placenta, the presence of vasa praevia should be examined and documented, if necessary as part of a further ultrasound examination (Merz et al. 2012; Schlembach et al. 2016).

This is also possible by abdominal sonography in case of a low-lying placenta with a sufficiently filled urinary bladder.

Tip

If placenta praevia is suspected, vaginal ultrasonography should be performed with an empty bladder (Heer et al. 2006). In contrast to digital examination, vaginal sonography is not contraindicated and does not lead to an increased risk of bleeding (Tikkanen et al. 2006). For a possible supplementary MRI, slice arrangements oriented sagittally to the vagina are recommended, as they best reveal the positional relationship of the placenta to the cervix.

Untypical Placental Location

Usually no clinical distinction is made between the exact location of anterior, lateral or posterior placenta. However, certain localizations are thought to be associated with certain fetal positions and attitudes, such as a breech presentation or an occiput posterior position with an anterior wall placenta (Fianu and Vaclavinkova 1978; Gardberg and Tuppurainen 1994). Recently, studies showed that a placenta that is neither anterior nor posterior in the second trimester is associated with an increased incidence of adverse obstetric outcomes (Fung et al. 2011; Seckin et al. 2015).

9.1.3 Placenta Accreta Spectrum (PAS)

As a consequence of a partial or total absence of the maternal decidua, which leads to direct contact between the chorion frondosum and the myometrium, there may be disturbances in placental implantation with abnormal adherence of the placental villi to the maternal myometrium.

Depending on the extent and depth of invasion of the placenta, different implantation disorders are distinguished (see following overview) (Belfort 2010).

Classification of Placenta Accreta Spectrum (PAS) (Belfort 2010)

- Placenta accreta: invasion up to the myometrial inner wall (81.6%)
- Placenta increta: invasion into the myometrium (6.6%)
- Placenta percreta: invasion to the uterine serosa or beyond the uterine borders (11.8%)

PAS occurs in 0.9% of all pregnancies and in 9.3% of pregnant women with placenta praevia. The risk of PAS increases with the number of cesarean deliveries (Table 9.1).

Eighty to ninety percent of all PAS present with placenta praevia (Garmi and Salin 2012). Additional predisposing factors are previous uterine surgeries (curettage, enucleation of fibroids), submucous fibroids, endometritis and placental abruption in the medical history (Garmi and Salin 2012).

Imaging

All pregnant women at increased risk for PAS should receive prenatal assessment for placental implantation (Merz et al. 2012; Schlembach et al. 2016). Diagnostic imaging includes sonographic assessment and MRI examination when appropriate. Prenatal detection of PAS is possible in approximately 50–80% (Soto and Hernández-Andrade 2015), and clinical suspicion should be followed by MRI examination—suspicion of PAS is the main indication for MRI examination of the placenta (Dekan and Linduska 2011; Soto and Hernández-Andrade 2015).

The lower uterine segment close to the cervix, a deep placental infiltration under the bladder and a possible cervical invasion in the case of placenta praevia can be examined more precisely by vaginal sonography than by abdominal sonography. Sonographic assessment is facilitated by a filled bladder in both approaches, as the bladder filling provides an ideal ultrasound window and the bladder wall is unfolded (Fuchs et al. 2008). Three-dimensional imaging of the findings can provide further hints, especially for ruling out the possibility of the placenta penetrating into the surrounding structures.

Table 9.1 Frequency of an abnormal placental implantation in relation to the number of previous C-sections and placenta praevia (Silver et al. 2006)

Cesarean section	PAS (%)	OR (95% CI)	Without placenta praevia (%)	With placenta praevia (%)
1 (primary)	0.2	–	0.03	3.3
2 (condition after C-section)	0.3	1.3 (0.7–2.3)	0.2	11
3	0.6	2.4 (1.3–4.3)	0.1	40
4	2.1	9.0 (4.8–16.7)	0.8	61
5	2.3	9.8 (3.8–25.5)	0.8	67
≥6	6.7	29.8 (11.3–78.7)	4.7	67

In the case of posterior wall placenta, the assessment of placentation is difficult due to the poorer ultrasound window without the urinary bladder as an anterior route or due to obstruction of the view by the fetus. Especially in these cases, the MRI examination can provide valuable information as an additional diagnostic procedure.

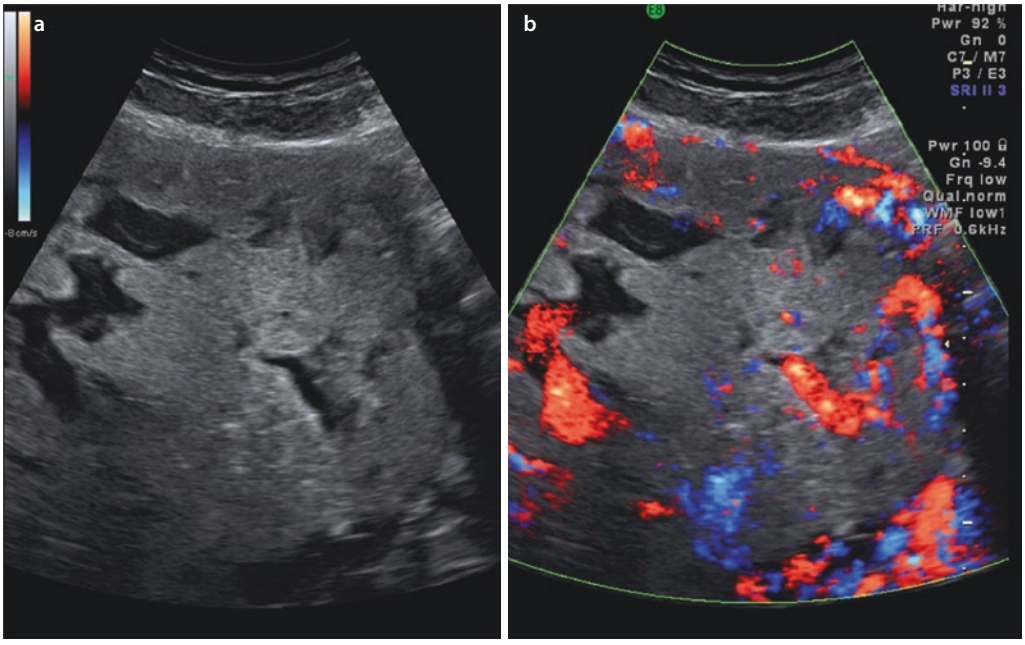
Sonographic Signs of PAS

PAS should be considered with the following findings (◘ Figs. 9.8 and 9.9) (Fuchs et al. 2008; Belfort 2010):

- Multiple lacunae in the placenta ("Swiss cheese")
- Loss of the hypoechogenic retroplacental zone
- Blood vessels or placental tissue break through the uteroplacental boundary
- Blurred demarcation between placenta and myometrium with retroplacental myometrium thickness < 1 mm
- Hypervascularization

Placental lacunae with turbulent flow (vascular bridges extending from the placenta into the myometrium) are the most characteristic sign of PAS with a sensitivity of 78–93%. The more lacunae to be visualized, the more likely an invasion disorder is present (Soto and Hernández-Andrade 2015). In contrast, the loss of the hypoechogenic retroplacental zone has a sensitivity of only 7% (Soto and Hernández-Andrade 2015).

Even though the diagnostic value of MRI has not yet been adequately clarified (Fuchs et al. 2008; Belfort 2010), an MRI examination should usually be performed in the third trimester in the case of sonographic



◘ **Fig. 9.8** **a**, **b** Placenta accreta spectrum (PAS). **a** Multiple lacunae ("Swiss cheese") and loss of the hypoechogenic zone; **b** Hypervascularisation

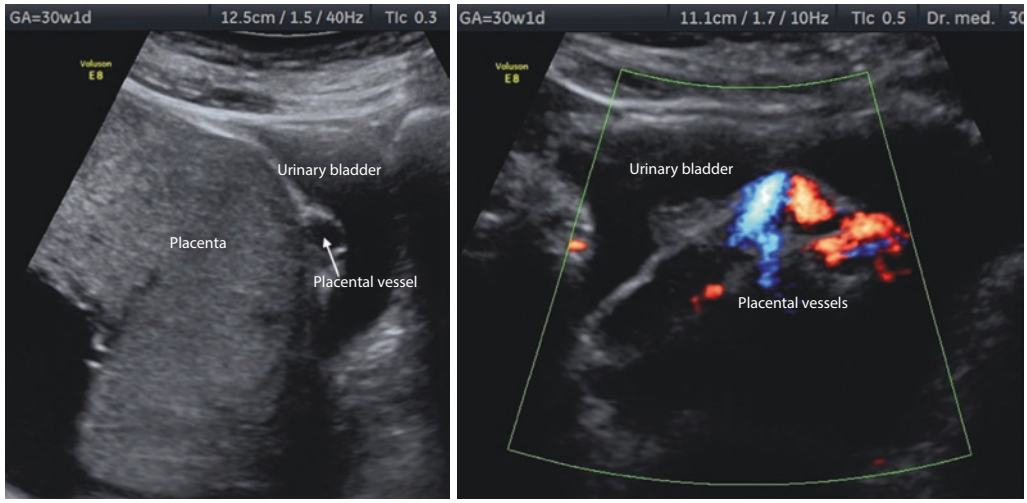


Fig. 9.9 Placenta accreta spectrum (PAS). Blood vessels break through the uteroplacental boundary to the urinary bladder

9

evidence of PAS in order to plan the delivery. The urinary bladder of the pregnant woman should be moderately full in order to better assess the bladder wall and to differentiate varices of the bladder wall from invasion or to avoid overestimation of invasion if the bladder is too full. It is essential that the MRI images are interpreted with knowledge of the sonographic examination/results and discussed jointly by radiologists and obstetricians. The large field of view of MRI makes it possible to visualize and assess the entire placenta. A detailed description of the structures involved (bladder, bowel, pelvic wall) is important for surgical planning (e.g. insertion of a ureteral stent).

MRI Criteria of PAS

The diagnostic criteria on MRI are similar to the sonographic features (Soto and Hernández-Andrade 2015; Lax et al. 2007; Bardo and Oto 2008):

- Uterine bulging: "protrusions" of the uterus
- Heterogeneous inhomogeneous signal infiltrating from the placenta into the myometrium
- Irregularly configured, hypointense lacunae in T2 weighting (blood flow/vascularization)—"Swiss cheese"
- Hypointense intraplacental bands in T2 weighting
- Focal invasion of the myometrium

According to Lax et al. (2007), some criteria important for sonography, such as thinned retroplacental myometrium, visualization of an exophytic mass and an irregular hyperechogenic border between uterus and bladder, are not useful for assessment by MRI.

A thinning of the myometrium or a tenting in the area of the urinary bladder are unspecific signs, which should, however, draw attention to look for further changes. In addition, a possible invasion into the parametria has to be evaluated. A placenta percreta can be described relatively reliably by MRI. Less pronounced forms of invasion of the placenta (accreta, increta) are more difficult to detect and differentiate (Figs. 9.10 and 9.11); however, this does not tend to influence the further procedure and the surgical technique. It must also be

borne in mind that different manifestations of invasion can occur in a pregnancy. A mere bulging of the uterus into the urinary bladder does not prove the presence of a placenta percreta, since the myometrium is thinned out and altered by scarring, especially in the case of previous C-section. Therefore, there is a risk of false-positive overestimation of invasion. In these cases, it is essential to demonstrate T2-weighted hypointense lacunar vascular connections between the uterus and the urinary bladder in order to prove the depth of invasion before surgery (Fig. 9.12). The intraplacental lacunae impose hypointense in the T2-weighted image; bizarre and irregular configurations allow differentiation from the typical placental septa formed during formation of the cotyledons. According to a meta-analysis, sonography and MRI in principle have comparable predictive value with regard to PAS (D'Antonio et al. 2014).

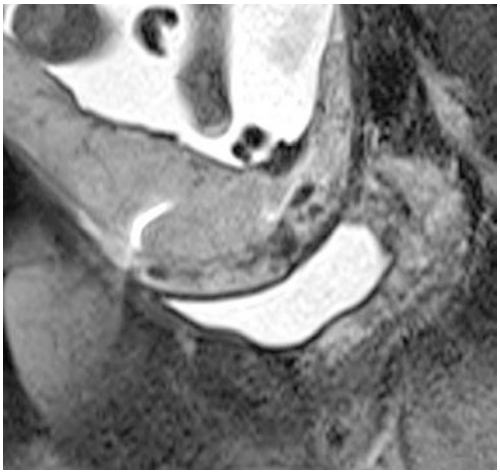


Fig. 9.10 Placenta increta. T2-weighted sagittal MRI with clearly hypointense caverns. The boundary to the myometrium is only clearly recognizable at the anterior wall

9.1.4 Echogenicity

The normal placenta appears relatively homogeneous. There are different types of abnormal echogenicity. Hypoechogenic lesions are the most common placental abnormalities and are usually diagnosed after the 25th week of gestation.

When examining the placenta, the complete placenta must be assessed in order to exclude placental lesions. If lesions are visualized, a Doppler sonographic examination should be followed.

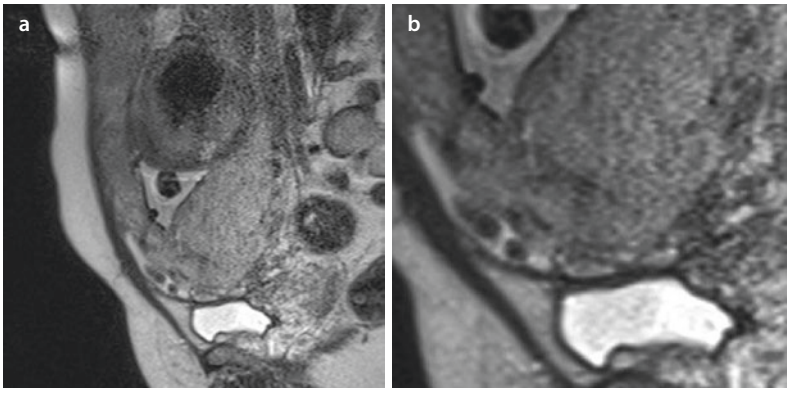


Fig. 9.11 **a**, **b** Placenta increta. T2w sagittal **a** with placenta praevia infiltrating the myometrium. Evidence of thick vacuolar lacunae in anterior wall region. Boundary to urinary bladder irregular, a breakthrough cannot be verified even in magnification **b**

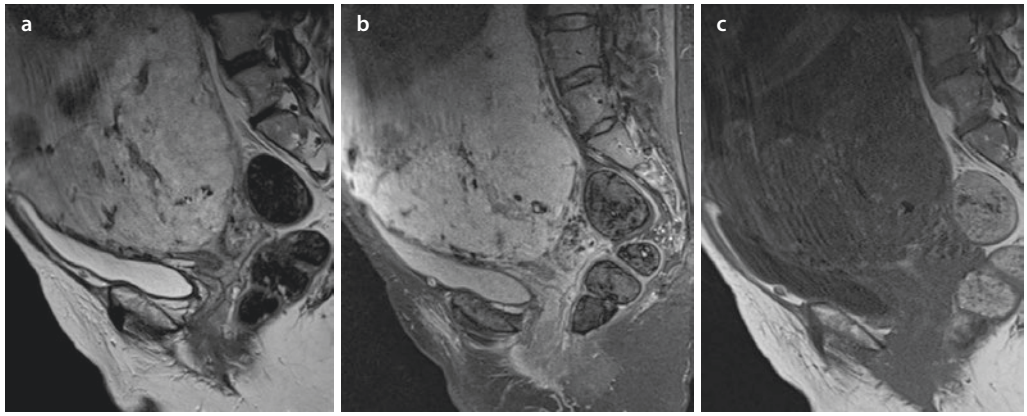


Fig. 9.12 **a–c** Placenta percreta. Evidence of T2w hypointense vascular structures in the border region towards the urinary bladder. These are best delineated in the T2-weighted sequence with fatty saturation, whereas in the T1w the vessels cannot be identified. Within the placenta in all sequences inhomogeneous signal and tubular vascular structures, the T1w hyperintense areas are most suggestive of fibrous/scar tissue in the placenta. **a** T2w HASTE; **b** TIRM; **c** T1w GRE

Placental Lacunae

Placental lacunae represent collections of maternal blood in the intervillous space (Kanne et al. 2005) and are the most common form of hypoechogenic areas (usually >2 cm) without villous structures.

They result from reduced intervillous blood flow, thrombosis, or fibrin deposition (Dekan and Linduska 2011) and are most commonly found in the late second and third trimester. The characteristic swirling venous flow can be visualized in real time. Placental lacunae (Fig. 9.13) should not be mistaken by the irregular, vascular lacunae typical for adherent placenta. Placental lacunae occur during pregnancy in 20–67% of all pregnancies (Bowman and Kennedy 2014) and have no clinical significance (Soto and Hernández-Andrade 2015; Thompson et al. 2002).

There are casuistics of MRI examinations reporting T1-hypointense and T2-isointense signal behavior—comparable to fresh blood (Morikawa et al. 2005).

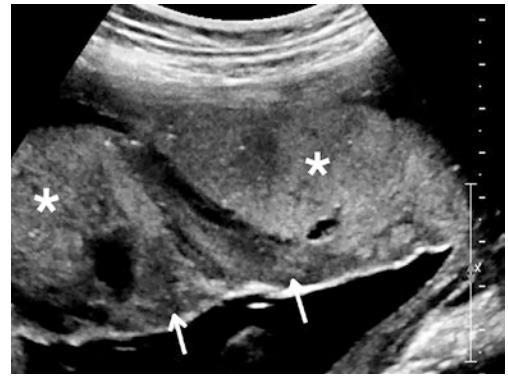


Fig. 9.13 Thick jelly-like placenta with placental lacunae (*asterisks*)

Septal Decidual Cysts

Septal cysts of the decidua (Fig. 9.14) arise from focal degeneration within the

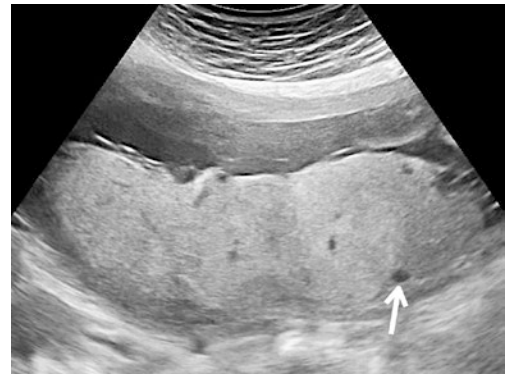


Fig. 9.14 Septal decidual cysts

maternal decidua. They are found in 20% of placentas at term. Septal cysts represent another form of hypoechogenic lesion, are usually <1–2 cm and have no clinical relevance (Dekan and Linduska 2011).

Placental Cysts of the Surface

True superficial placental cysts are located on the fetal side of the placenta, near the base of the umbilical cord. Most often, they are simple cysts with echogenicity corresponding to the amniotic fluid. The prevalence is 2–7%, they are usually small and are not detected sonographically. Usually they do not cause any problems, rarely association with fetal growth restriction (FGR) is reported. If there are more than three cysts or if cysts are >3 cm, especially near the base of the umbilical cord, regular biometry should be performed and fetal cardiac function should be monitored (Raga et al. 1996; Brown et al. 2002).

Echogenic Cystic Lesions (Placental Bed Infarction)

These lesions, 1–2 cm in diameter, have a central fluid-filled space and usually present clearly (Fig. 9.15). Echogenic cystic lesions can be distinguished from other lesions by their hyperechogenic margin, which represents perivillous fibrin deposition, and their irregularly notched border. In addition, unlike placental lacunae, there is no flow on color Doppler.

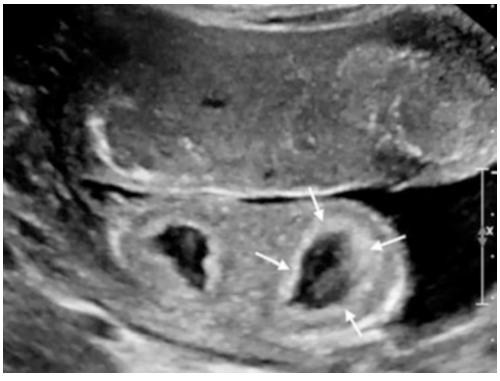


Fig. 9.15 Echogenic cystic lesions

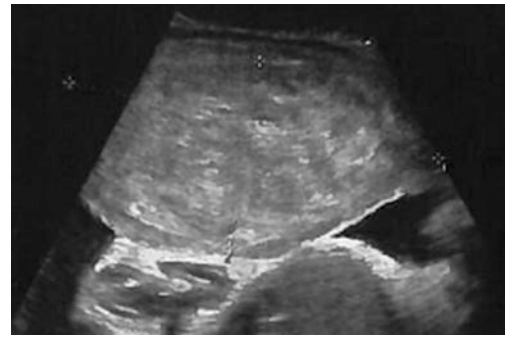
The etiology of echogenic cystic lesions is unclear. Approximately 10–15% of all pregnant women with pregnancy complications, or 0.09% of all pregnant women form pronounced perivillous fibrin deposits, so-called Gitter infarcts. According to recent research, these are intervillous thrombi, which consist of coagulated maternal blood in the intervillous space surrounded by compressed or infarcted villi (Soto and Hernández-Andrade 2015; Harris et al. 1990, 1996). They are associated with adverse pregnancy outcome (habitual abortions, IUFD, FGR, preeclampsia, preterm delivery) (Proctor et al. 2010). The triad of echogenic cystic lesions, abnormal uterine artery Doppler and abnormal placental shape is usually indicative of severe placental impairment and can predict perinatal death with a probability of 52% (Viero et al. 2004).

Placental Infarction

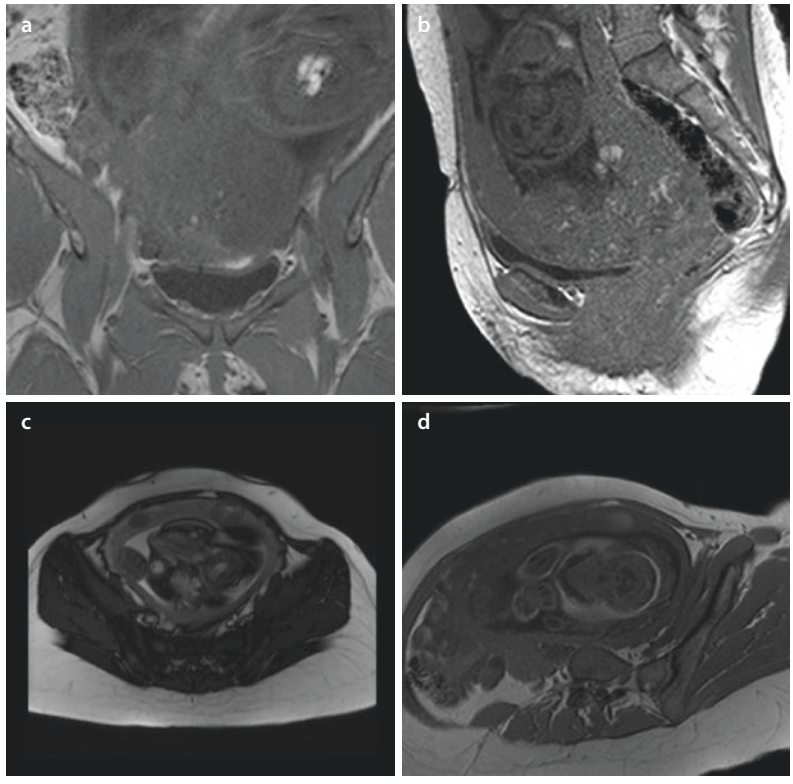
Placental infarctions are detectable in approximately 20% of all uncomplicated pregnancies and in 40% (of mild) and 70% (of severe) preeclampsia (Moldenhauer et al. 2003; Krielessi et al. 2012). In 39% of all pregnant women with preeclampsia, the placenta is more than 5% infarcted (Vinnars et al. 2011). Placental infarctions present as echogenic or even anechogenic avascular areas and result from lack of dilatation or thrombotic occlusion of the spiral arteries, increased perivillous fibrin deposition, and disruption of fetal circulation due to fetal thrombotic vasculopathy (Soto and Hernández-Andrade 2015). On MRI, ischemic infarctions of the placenta can be delineated as hyperintense in T2 weighting and diffusion weighting (DWI) trace image; hemorrhagic and thrombotic infarctions are hyperintense in T1 weighting (Linduska et al. 2009).

Placental Hematoma

Hematomas present sonographically as hypo- to anechogenic (acute or chronic) preplacental (to the fetus), retroplacental (to the mother; ◘ Fig. 9.16), retrochorial or retroamniotic zones distributed marginally along the placental border or intraplacentally. Subacute isoechogenic hemorrhage can be verified by displacement effects, for example, on perfusion assessment. On MRI examination, hematomas may have variable signal intensities, depending on the age and composition of the hemorrhage (◘ Fig. 9.17). Subacute hemorrhages should be better differentiated by MRI tomography than by sonography; however, any therapeutic decision will be determined by clinic features and not by MRI (Masselli et al. 2011). In T1 imaging, hematomas can be delineated hyperintensely, whereas in the more T2-weighting blood-sensitive gradient echo (GRE) sequence, they are hyopinten-



◘ **Fig. 9.16** Retroplacental hematoma (11.2 cm × 9.5 cm)



◘ **Fig. 9.17** **a–d** Placental hematomas/hemorrhages on MRI. **a**, **b** T1w images (T1 fl2d): the hyperintense changes within the inhomogeneous placenta correspond to hemorrhages (**a**: coronary, **b**: sagittal). **c**, **d** T2w and T1w axial images: T2w-hypointense and T1w-hyperintense circumscribed changes, at 36th week of gestation with circumscribed hemorrhages within the placenta (**c**: T2 Haste tra; **d**: T1 fl2d tra)

Table 9.2 Ischemic and hemorrhagic pathology on MRI (Dekan and Linduska 2011)

	T1w	T2w	GRE	DWI	Localization	Morphology
Ischemia	–	+	–	+	Intraplacental	Diffuse/delineated
Hemorrhage	+	–	+	+	Intraplacental	Diffuse/delineated
Subchorial hemorrhage	+	–	+	+	Subchorial	Delineated
Intervillous hemorrhage	+	–	+	+	Intraplacental	Round
Intervillous thrombi	–	+	±	±	Intraplacental	Delineated
Retroplacental hematoma	+	–	+	+	Retroplacental	Delineated

+ *h*yperintens, – hypointens, *GRE* gradient echo, *DWI* diffusion weighting

sive (Table 9.2) (Dekan and Linduska 2011). Randomly verified blood collections without clinical symptoms are mostly venous in origin and should only be controlled by follow-up examinations.

Hematomas are associated with adverse pregnancy outcomes (habitual abortions, IUFD, placental abruption, FGR, preterm birth), but lack of uniform standardized definition criteria makes it difficult to make a valid statement about the incidence of pregnancy complications (Soto and Hernández-Andrade 2015).

Breus' Mole

It is a rare, distinct (>50% of placental area) subchorial hemorrhage of unclear etiology, first described by Breus in 1892 (Jha et al. 2016). Due to massive hemorrhage, there is a high risk of adverse pregnancy outcome (FGR and in up to 50% IUFD) (Alanjari et al. 2013).

The sonographic picture is identical to that of hematomas: Initially, hyperechogenic areas without blood flow are seen, which take on a heterogeneous appearance with increasing clot formation.

Jelly-like Placenta

The jelly-like placenta is characterized by inconsistent echogenicity and anechogenic spaces (Figs. 9.18 and 9.19). It moves like

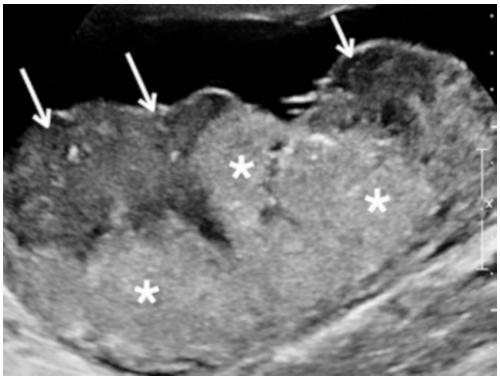


Fig. 9.18 Jelly-like placenta

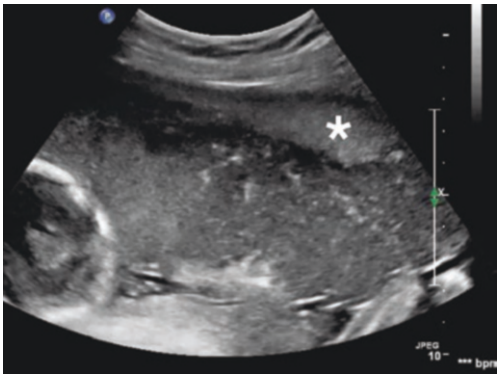


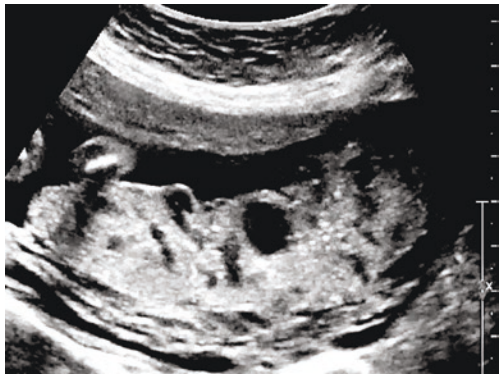
Fig. 9.19 Thickened jelly-like placenta, anhydramnion

jelly in response to abdominal pressure (Jauniaux et al. 1990). Placental thickness is usually >95th percentile (Jauniaux et al. 1994). Most jelly-like placentas are located laterally, close to the fundus (Raio et al. 2004). This location is often associated with poor perfusion, preeclampsia and FGR (Kofinas et al. 1989). Jauniaux et al. were the first to describe the triad of abnormal maternal blood counts, pathological uterine Doppler and small jelly-like placenta with anechogenic lakes in FGR and hypertension (Jauniaux et al. 1994).

Mesenchymal Dysplasia of the Placenta

Placental mesenchymal dysplasia was described in 1991 as a placental vascular abnormality with diffuse hyperplasia of the villi (Moscoso et al. 1991). With a thick placenta displaying hypoechogenic areas, the ultrasound findings characteristic of this entity are similar to those of a hydatidiform mole (◘ Fig. 9.20). However, in contrast to partial moles, most fetuses with placental mesenchymal dysplasia have an unremarkable karyotype, and ßhCG levels remain normal throughout pregnancy.

In order to prevent non-indicated abruptions, placental mesenchymal dysplasia must be clearly distinguished from hydatidiform moles (Parveen et al. 2007). If a suspected diagnosis of placental mesenchymal dysplasia is made after ultrasound diagnosis and genetic testing, the affected patients must be treated as high-risk pregnant women, since placental mesenchymal dysplasia is associated with fetal growth restriction, intrauterine fetal death and other chromosomal abnormalities.



◘ **Fig. 9.20** Mesenchymal dysplasia of the placenta

9.1.5 Maturation of the Placenta

Sonographically, the degree of maturity of the placenta can be divided into four grades according to Grannum et al. (1979) (see following overview; ◘ Fig. 9.21).

Sonographic Classification of Placental Maturation (According to Grannum et al. 1979)

- Grade 0: homogeneous parenchyma, smooth bordered chorionic plate (approx. 12–30 weeks of gestation)
- Grade I: slightly wavy chorionic plate, single parenchymal echo enhancements (approx. 30–32 weeks of gestation)
- Grade II: elongated echo enhancements from the chorionic plate towards the basal plate, echo-rich structures in the parenchyma (32–35 weeks of gestation)
- Grade III: chorionic plate interrupted (compartmentalization), cotyledons recognizable, hyperechogenic structures in the parenchyma, garland-shaped pattern (>36th weeks of gestation)

The degree of maturity of the placenta is based primarily on the presence and distribution of calcifications from calcium deposits. In the late 1970s, Grannum et al. developed a method for determining placental maturity to predict fetal development (Grannum et al. 1979). According to this method, Grannum 0 corresponds to a less

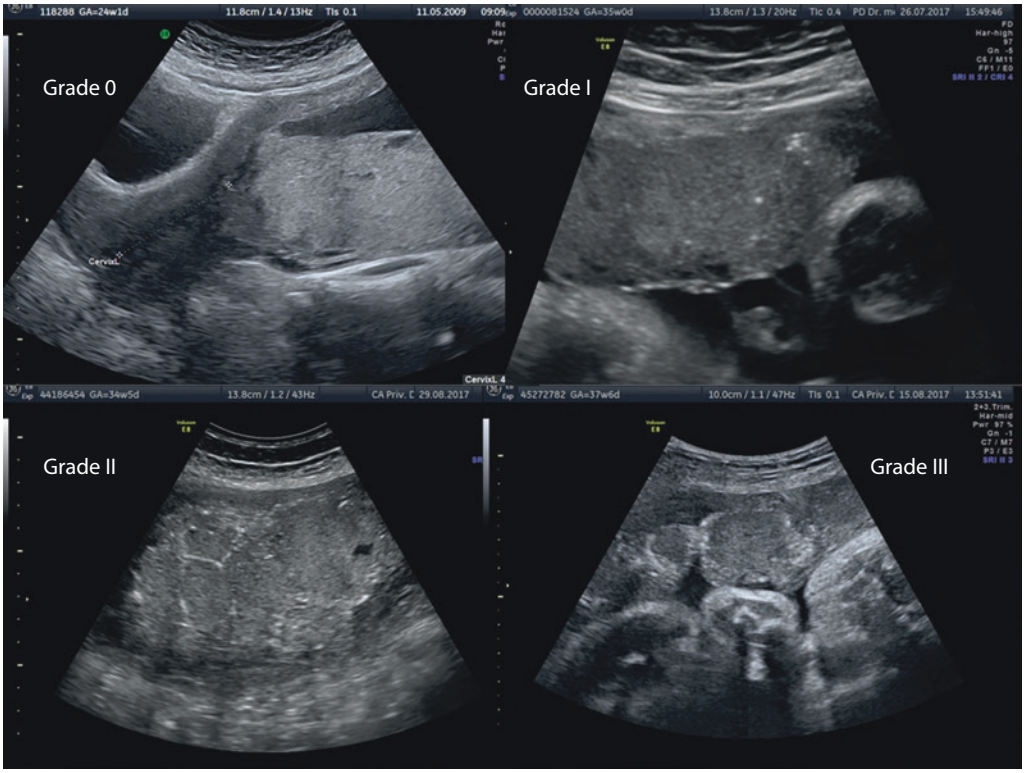


Fig. 9.21 Sonographic representation of placental maturity grades 0–III

mature placenta, whereas Grannum III corresponds to a very mature placenta. The assessment of placental echogenicity (decreased or increased) can be used in the identification of risk constellations (Jauniaux et al. 1990; Raio et al. 2004).

Analogous to the sonographic classification according to Grannum, MRI can also determine the degree of maturity of the placenta. T2-weighted sequences show the placenta in early pregnancy to be moderately hyperintense and thus well demarcated from the myometrium. If the placenta is relatively homogeneous in T1 and T2 weighting in the first and early second trimester, it becomes inhomogeneous with irregular surface and lobulated internal structures (cotyledons) in the further course (Dekan and Linduska 2011; Blaicher et al. 2006).

However, the assessment of placental maturity has lost importance in recent years due to the weak correlation with a poor perinatal outcome and especially due to the high subjectivity of the method (Sau et al. 2004; Moran et al. 2011), especially since Doppler sonography is a much better method for assessing the fetoplacental unit.

9.1.6 Size and Shape

The placenta is usually round to oval with a wide variability (Yampolsky et al. 2008). Irregular shapes are mainly determined by localization, atrophy and implantation.

Accessory Placenta (Placenta succenturiata)

In about 8%, an accessory lobe (placenta succenturiata) is present, which is connected to the main placenta by vascular bridges and caused by partition. The most common variant is placenta bilobata (Meizner et al. 1998; Soto and Hernández-Andrade 2015). A placenta succenturiata can be the cause of fetal hemorrhage (due to rupture of vasa aberrantia—especially during rupture of the fetal membranes) or of placental retention (Suzuki and Igarashi 2008). Therefore, a careful search for aberrant vessels (especially vasa praevia) should be carried out, especially in the case of accessory placentas in the lower uterine segment.

Accessory placentas should always be excluded sonographically, as they may remain in utero and thus lead to postpartum complications (bleeding).

Placenta circumvallata

Placenta circumvallata is a morphologically abnormal placenta in which the transition from membranous (chorion laeve) to villous chorion (chorion frondosum) is not at the margin of the placenta but at some distance from it (Fig. 9.22) (Scott 1960). The chorionic plate on the fetal side, from which the villi originate, is smaller than the basal plate on the maternal side of the placenta. In this constellation, placental tissue is located outside the boundary of the chorionic plate, giving rise to the name "placenta extrachorialis." A double layer of amnion and chorion with necrotic villi and fibrin forms an annular placental rim. Difficult to detect prenatally, one may see sonographically irregular, raised placental margins as well as a peripheral ring of chorionic tissue, which appears as an echodense ridge (Soto and Hernández-Andrade 2015; McCarthy et al. 1995; Harris et al. 1997; Suzuki 2008; Elsayes et al. 2009). In three-dimensional ultrasound, this feature appears like a tire (the "tire sign") (Arlicot et al. 2012). The incidence of placenta circumvallata is 1–2%, and it is associated with an increased rate of perinatal complications, such as prematurity, oligohydramnios, premature rupture of membranes, pathological CTG, placental abruption, and intrauterine fetal death (IUFD).

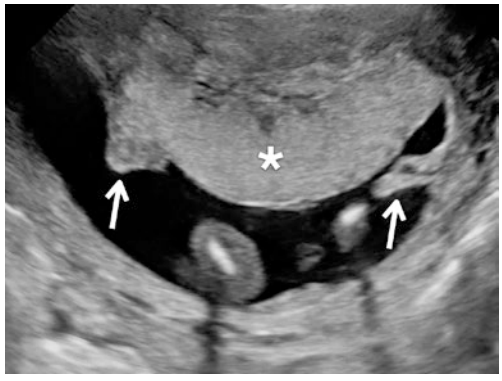


Fig. 9.22 Placenta circumvallata

Placenta membranacea/Placenta diffusa

Placenta membranacea/placenta diffusa represents a rare placental anomaly (incidence approximately 1/20,000 births, 1/185 for placenta praevia) (Soto and Hernández-Andrade 2015), possibly associated with placenta accreta (Pereira et al. 2013). The amnion is completely or partially covered with a thin membrane of villous tissue (Dekan and Linduska 2011; Soto and Hernández-Andrade 2015). The risks reported here are pre- and postpartum hemorrhage, late abortions, and fetal growth restriction (FGR) with an increased risk of IUFD (Soto and Hernández-Andrade 2015). Placenta membranacea should be considered if imaging shows a thin layer of placental tissue over most of the uterine cavity.

9.1.7 Placental Biometry and Volumetry

Biometry and volumetry of the placenta have been suggested as potentially useful methods for estimating perinatal risks (Elsayes et al. 2009).

For area and volume calculations, a circular placental shape is generally assumed. However, in conventional ultrasound examination, two-dimensional measurements (thickness, diameter of the placenta) and area calculations derived from them can be achieved with significant variability of the measured values. Placental volume can also be determined using 3D sonography and MRI. Sonographic volume calculation is performed either by means of multiplanar measurements or special algorithms such as VOCAL (Virtual Organ Computer-Aided Analysis) (Kalache et al. 2003).

The diameter of the placenta is approximately 18–20 cm, and the normal placental thickness is approximately 2–4 cm (Kaplan 2008; Lee et al. 2012) measured centrally or at the base of the umbilical cord if it is centrally located. In daily clinical routine, the placental thickness is only estimated subjectively and, if it appears inconspicuous, not measured.

Tip

It is recommended to measure placental thickness at the site of placental cord insertion.

Placental location and, if possible, gestational age must be considered when evaluating placental thickness. If the umbilical cord insertion is approximately central (90% of placentas are circular and have a central umbilical cord insertion), this will provide a correct measurement in most cases. If the umbilical cord insertion is marginal, this method will often measure a placenta that is too thin (Lee et al. 2012).

In normal pregnancy, the placenta shows a continuous growth in thickness until about the 37th week of gestation, whereat the thickness of the placenta (in mm) corresponds to the gestational age in weeks (Schlensker 1971). Anterior wall placentas are about 0.7 cm thinner than posterior wall and fundal placentas. Anterior wall placentas in the second trimester of >3.3 cm thickness and posterior wall placentas >4.0 cm thickness are considered "abnormal" (Soto and Hernández-Andrade 2015; Lee et al. 2012; Hoddick et al. 1985), and a thickness > 5 cm is considered pathological (Elchalal et al. 2000). Placentas that are too thick, as well as placentas that are too thin, indicate an increased perinatal risk as non-specific findings (Jauniaux et al. 1994).

Postpartum examination of placentas in case of extreme preterm birth with FGR revealed that more than 50% have abnormalities in size, shape, and umbilical cord insertion (Pomorski et al. 2012; Walker et al. 2012). Studies of placental morphology show that placentas from women with preeclampsia tend to be oval rather than round and have reduced surface area (Burton et al. 2010). Marginal and velamentous cord insertions are associated with smaller placentas and smaller newborns (Vinnars et al. 2011).

Thick Placenta

Several studies described an association between increased placental volume and adverse pregnancy outcomes such as placental abruption, FGR, hypertension, neonatal acidosis and fetal death (Jauniaux et al. 1994; Raio et al. 2004; Elchalal et al. 2000; Eskild et al. 2009; Dombrowski et al. 1992; Proctor et al. 2009; Cooley et al. 2011; Porat et al. 2013; Miwa et al. 2014). However, at the time of diagnosis of a thick placenta, fetal and maternal blood flow, an early sign of abnormal conditions, was not different between the two groups (Arabin et al. 1992). This suggests that the ultrasonographically

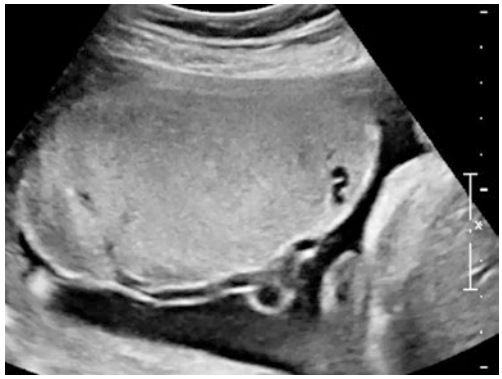


Fig. 9.23 Thick placenta

detected thick placenta (Fig. 9.23) corresponds to the latent phase of a placental dysfunction.

A possible explanation for the increase in size due to placental dysfunction could be the compensatory proliferation and edema of the placental villi (Raio et al. 2004; Fox and Elston 1978; Todros et al. 1999a). In other words, the thick placenta would be the result of compensatory hyperplasia of certain placental areas that are not affected by inadequate uteroplacental blood flow.

Another possible explanation could be the loss of anchoring villi. In a normally developed placenta, the distal tips of the placental villi are anchored to the decidua, providing a solid structure, so that pressure in the maternal vessels cannot affect the surface of the fetal chorionic plate. This is consistent with the observation that a proportion of thick placentas are associated with a marked arrest in placental development, which is characterized by hypoplasia of distal villi (Fig. 9.24) (Macara et al. 1996). The hypoplastic villi are the first sign of a disturbance in the formation of the villi responsible for gas exchange when angiogenesis has stopped (Macara et al. 1996). Areas with fewer villi are therefore filled with maternal blood and present on sonography as slowly moving even beyond the basal plate. These placentas appear to shrink after birth as greater volumes of maternal venous blood likely flow out of the intervillous space via the disrupted uteroplacental veins (Porat et al. 2013).

Nevertheless, three-dimensional placental volume measurement cannot be used to screen for FGR. In high-risk cases, the placental volume consists mainly of maternal blood and not of functional placental villous tissue. In this respect, the sole assessment of placental size and shape as well as echogenicity by 2D sonography is more informative for serious abnormalities in placental development.

Small Sized Placenta

In sonographic volume assessment, the main limitation of the method is the increasing size of the placenta with increasing gestational age and the change in the shape of the pregnant woman's abdomen. From the late second trimester onwards, it is difficult to capture the complete placenta with the volume transducer (Hata et al. 2011); hence, the scientific focus of placental volumetry has been on the value of the method in detecting or predicting pregnancy complications. Small placentas are associated with perinatal complications. A prospective study of 712 women showed that low placental volume in the second trimester precedes low birth weight. This association already exists in the first trimester, which could be shown by means of 3D volume measurement (Hafner et al. 2001a, b).

In particular, the working group around Hafner investigated the association of placental volume in the first trimester and found a significant association with preeclampsia developing later (Hafner et al. 2006). Other research groups (Odeh et al. 2011a; Odibo et al. 2011; Rizzo et al. 2012) were unable to establish an association between placental volume and pregnancy complications, so that ultimately the determination of placental volume as a prognos-

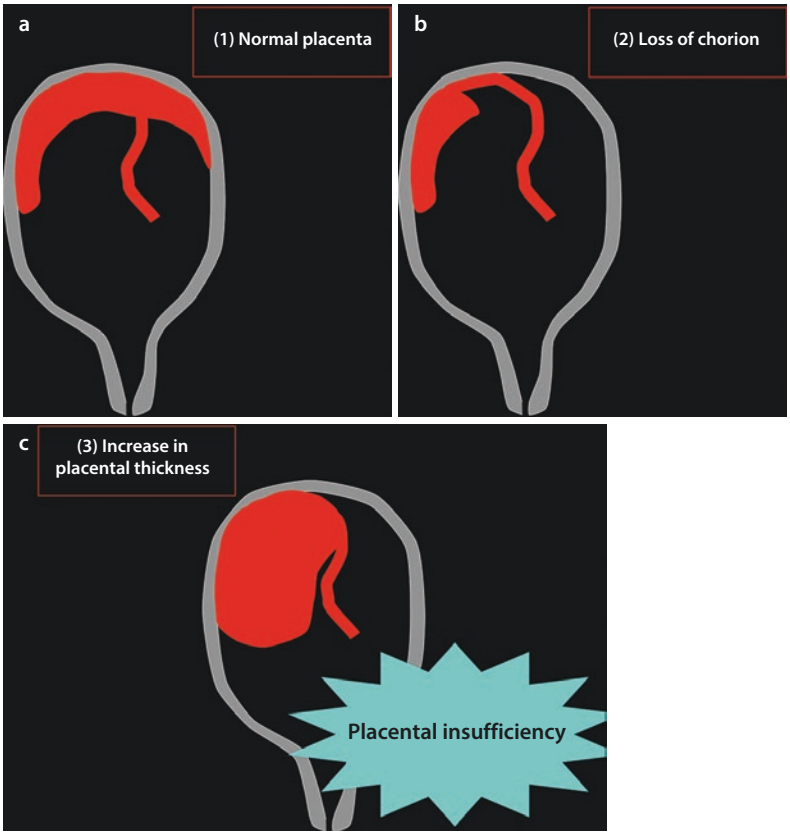


Fig. 9.24 **a–c** Scheme for the development of a thick placenta

tic marker for pregnancy complications has not been established in clinical practice.

Placental Abruption

The diagnostic value of an ultrasound examination for premature placental abruption is limited; the diagnosis is usually made clinically (abdominal pain, contractions, and possibly bleeding).

A detachment of >50% increases the risk of IUFD (Soto and Hernández-Andrade 2015). Ultrasound or MRI may be relevant for classification of location (subchorial, retroplacental, retroamnial) in less pronounced hematomas/detachments (Nyberg et al. 1987).

The acute hemorrhage presents sonographically hyperechogenic compared to the surrounding placental tissue. With increasing age of the hematoma the area becomes increasingly hypoechogenic (Nyberg et al. 1987).

9.1.8 Summary

Ultrasound examination of the placenta is increasingly used to detect high-risk pregnancies. Several studies show a clear association between a sonographically abnormal placenta and poor perinatal outcome. Abnormal placental morphology may imply altered placental structure, which may lead to poor perinatal outcome.

9.2 Doppler Sonography/ Functional Diagnostics

Dietmar Schlembach

The placenta supplies the fetus with nutrients. As an endocrine organ, the placenta produces a large number of hormones that influence the mother and the fetus, but also placental development itself. The proper formation and development of the placenta and the utero- and fetoplacental vascular systems are thus essential for adequate placental function, especially the supply of oxygen and nutrients to the fetus. Disturbance of placental function (including disturbance of utero- or fetoplacental perfusion) can lead to complications resulting in increased maternal and perinatal morbidity and mortality (fetal growth restriction [FGR], intrauterine fetal death [IUFD], preeclampsia, fetal malformations) (Graf 2008; Pasca and Penn 2010).

The function of the placenta can be disturbed by various mechanisms (e.g. disturbance of gene expression, infections, premature birth) with immediate maternal and fetal/neonatal consequences and possible consequences in the long-term outcome (Pasca and Penn 2010).

The gold standard for evaluation of the utero- and fetoplacental circulation is Doppler sonography.

MRI scans can also be used to visualize vascular placental pathologies (Messerschmidt et al. 2011), but this is not currently of any value in clinical practice.

The basic prerequisite for a sufficient evaluation of the findings is an exact knowledge of the morphology, physiology and pathology of the placenta (► Chaps. 3 and 4) as well as of the feeding and draining vessels.

9.2.1 Placental Vascular System

With two circuits—uteroplacental and fetoplacental—the placenta is an extremely complex organ that reaches its maximum functional capacity before birth. Disturbances in normal development can have a drastic effect on fetal well-being. Over the course of nine months of development, the placenta forms a vascular network approximately with a length of 500 km and a surface of 12–14 m^2 through vasculogenesis and angiogenesis (Burton and Fowden 2015). Control of this vascular network and placental circulation occurs in the absence of autonomic nervous regulation through the release of local factors. The muscular arterial vessels of the villi constitute the primary resistance flow area of the placenta, the tone of which is modulated by nitric oxide (NO), among other factors (Myatt 1992). Through this complex regulation, blood flow in the placenta is matched to maternal perfusion to ensure optimal placental function (Burton and Fowden 2015).

The placenta is characterized by a special cell type—the trophoblast. The trophoblast differentiates into the villous and extravillous trophoblast. The villous trophoblast forms the villous tree of the placenta, while the extravillous trophoblast invades the placental bed and stimulates remodeling of the spiral arteries, and is thus crucial for adequate uteroplacental blood flow and fetal delivery of oxygen and nutrients (Burton and Fowden 2015; Sheppard and Bonnar 1981; Everett and Lees 2012; Osol and Moore 2014).

Utero- and fetoplacental perfusion depend on blood pressure, vascular resistance and blood viscosity. Various factors, e.g. drop of blood pressure, changes in vascular tone, vascular/endothelial lesions or placental infarctions, intervillous thrombosis and placental hematoma can affect uteroplacental perfusion. Fetoplacental perfusion may be affected, for example, in labor (con-

striction of vascular lumen and increase in resistance), thrombosis or disruption of the degree of maturation of the villi, and pathological changes in the placenta (Giles et al. 1985; Voigt and Becker 1992; Hitschold et al. 1993; Krebs et al. 1996; Todros et al. 1999ab; Shilling et al. 2014; Baron et al. 2015).

Fetoplacental Hemodynamics

Via the umbilical vein, 20–30% of the oxygenated and nutrient-rich blood reaches the liver and 70–80% reaches the heart via the ductus venosus (Chaoui et al. 2014) and the fetal circulation. From there, the fetal oxygen-depleted blood flows back into the placenta via the umbilical arteries.

> The blood flow in the umbilical arteries is a measure of the size of the perfused fetoplacental vascular tree.

The larger the vascular tree, the lower the vascular resistance, i.e. the greater the blood flow. The size of the fetoplacental vascular tree (fetal intravillous blood volume) is the product of villous vascularization and placental weight or volume (Graf 2008; Giles et al. 1985; Hitschold et al. 1993; Krebs et al. 1996; Todros et al. 1999ab).

With normal placental histology (vascular tree and villi) and normal placental weight, unremarkable blood flow patterns can be visualized in the umbilical arteries unless additional factors are present that impair perfusion, such as compression of the umbilical cord (Graf 2008).

Uteroplacental Hemodynamics

The uterine arteries arise from the internal iliac arteries and divide at the level of the inner cervical os into an ascending and a descending artery. From the ascending arteries, on the lateral wall of the uterus, arise the arcuate arteries, which form a vascular network with vessels of the contralateral side and from which arise the radial arteries. The radial arteries pierce the myometrium and divide into basal arteries and spiral arteries at the junction with the endometrium or decidua. The spiral arteries pass through the decidual basal plate (Graf 2008).

Before the eighth week of gestation, invasive endovascular (now: endoarterial) trophoblastic plugs occlude the spiral arteries, while minimal hemochorial perfusion exists (Jauniaux et al. 2000), and the embryo is supplied by "histiotrophic" nutrition—secreted by uterine glands (Burton et al. 2002). As gestational age advances, the spiral arteries open and intervillous circulation is complete by the end of the first trimester (Burton and Fowden 2015; Burton et al. 2010). Maternal blood reaches the intervillous space via the spiral arteries of the uterine arteries, circulates around the chorionic villi, and drains peripherally via sinusoidal veins in the decidual septa and via the marginal sinusoids in the placental margin (Graf 2008; Burton and Fowden 2015).

Remodeling of the spiral arteries results in low resistance wide vessels that carry large volumes of blood from the maternal vessels into the intervillous space without high vascular resistance (Osol and Moore 2014; Burton and Fowden 2015; Redman and Sargent 2005). Failure to remodel the spiral arteries results in altered perfusion of the placenta and fetus and an increased incidence of pregnancy complications (FGR, preeclampsia) (Redman and Sargent 2005).

Disorders of Utero- and Fetoplacental Blood Flow

A large number of studies have demonstrated the association of placental insufficiency and pathological utero- and fetoplacental flow patterns. These changes are closely associated with pregnancy complications and adverse neonatal outcome and often proceed in a temporary cascade (Ferrazzi et al. 2002; Baschat et al. 2007). The vessels important for diagnosis and evaluation are first of all the uterine arteries and the umbilical arteries, as these vessels

represent the maternal and fetal circulation, respectively. Disturbances in placentation are reflected in increased resistance indices in the uterine and/or umbilical vessels (Figs. 9.25 and 9.26).

Impaired uteroplacental perfusion is diagnozed when the mean pulsatility index (PI) is above the 95th percentile and/or a persistent notch is present. It significantly increases the risk of placental dysfunction in both low- and high-risk collectives (Hernandez-Andrade et al. 2002; Cnossen et al. 2008; Gómez et al. 2008).

At term, increased resistance in the uterine arteries is associated with an increased risk of adverse perinatal outcome, independent of fetal weight (Monaghan et al. 2018). However, the presene of an impaired uteroplacental perfusion does not allow to distinguish between the different placenta-associated pregnancy complications (preeclampsia, placental abruption, FGR, IUFD), but aids in the differential diagnosis of FGR (Kehl et al. 2017).

Estimation of the uteroplacental resistance in first and second trimesters is currently being promoted as a potential screening method for the detection of early preeclampsia and FGR (Cnossen et al. 2008; Bahado-Singh and Jodicke 2010; O'Gorman et al. 2016a).

While the negative predictive value is excellent in the presence of an unremarkable Doppler (Bahado-Singh and Jodicke 2010), the sensitivity and positive predictive value is not suitable for use in general screening as a single marker, which is why combination with other markers is recommended (Gabbay-Benziv et al. 2016; O'Gorman et al. 2016a, b, 2017a, b; Yücel et al. 2016).

With increasing uteroplacental perfusion disturbance, the fetoplacental compartment is also compromised.

The first sign is abnormalities in the flow pattern of the umbilical arteries (Baschat et al. 2001). The assessment is quantitative (measurement of the resistance indices) on the one hand and qualitative with the assessment of the end-diastolic flow on the other. Absent end-diastolic flow occurs when approximately 60–70% of the villous vascular tree is defective (Vergani et al. 2005); further deterioration may result in reversed end-diastolic flow in the umbilical arteries.

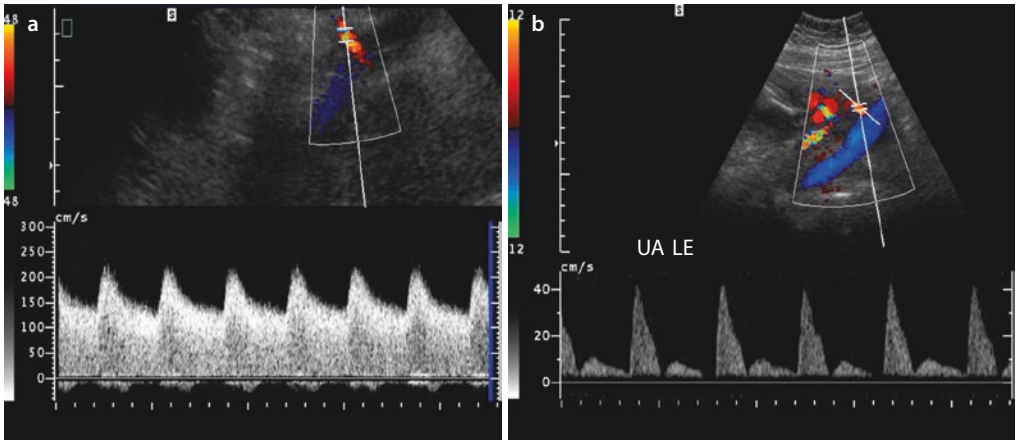


Fig. 9.25 **a**, **b** Doppler sonography of the uterine arteries: normal **a** and pathological **b** flow pattern of the uterine artery. *UA LE* left uterine artery

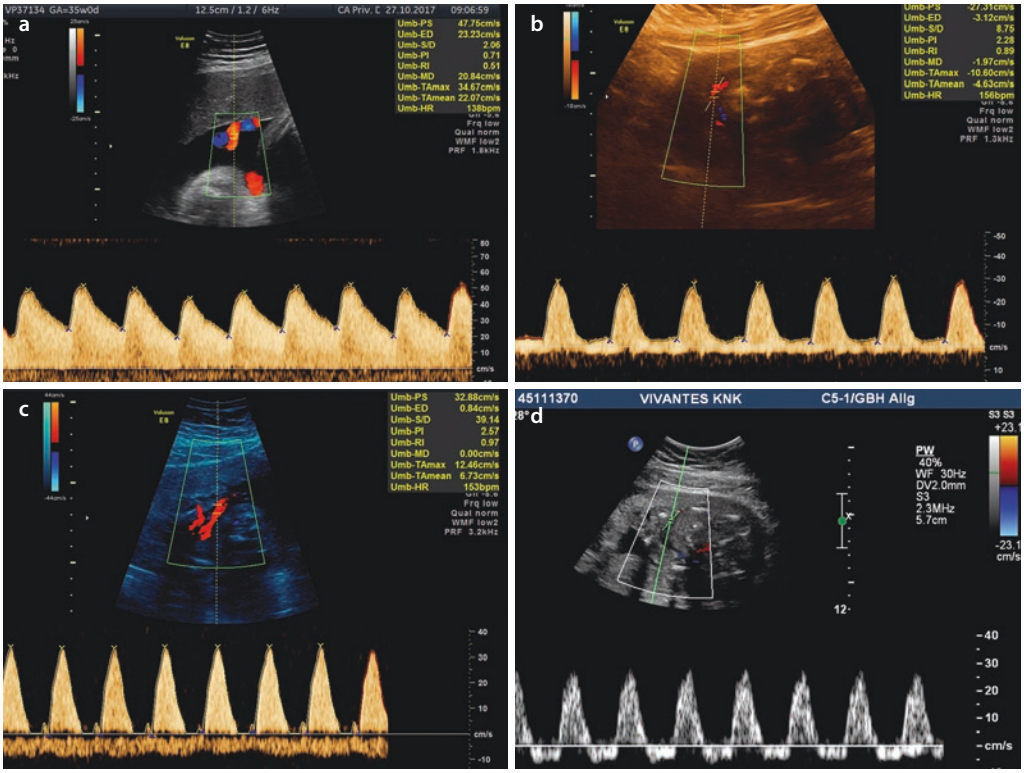


Fig. 9.26 **a–d** Doppler sonography of the umbilical arteries. Normal flow pattern of the umbilical artery **a** Doppler sonographic changes in the blood flow of the umbilical artery during fetal growth restriction: **b** decreased end-diastolic flow and increased pulsatility index; **c** absent end-diastolic flow (zero-flow); **d** reversed end-diastolic flow.

Placental Function

Assessment of placental function as the primary source of nutrition for the developing fetus is of paramount importance. In the past, various parameters (weights, measures and ratios) have been developed to assess placental function. However, all these parameters reflect the function of the placenta only very unsatisfactorily. In contrast, the pathological-anatomical examination of the placenta, umbilical cord and fetal membranes is more informative (Graf 2008).

Recent studies use magnetic resonance imaging (MRI) methods to investigate oxygenation and placental metabolism—in addition to blood flow assessment. Functional MRI examination offers the possibility to study vascularization, oxygenation and metabolism by means of different enhancement processes (Javor et al. 2013; Siauve et al. 2015; Mourier et al. 2017). However, since contrast imaging using gadolinium is contraindicated, alternative contrast agents must be used (Siauve et al. 2015).

Placental Blood Flow

Evaluation of placental blood flow by (three-dimensional) power Doppler ultrasonography has been proposed as a potentially useful tool for predicting pregnancy complications (Odeh et al. 2011ab). Inconsistent results and especially the low reproducibility

(Cheong et al. 2010) are reasons why this method has not found its way into routine diagnostics.

Moore et al. (2000a) reported indirect measurement of placental blood flow using magnetic resonance imaging (MRI). The technique called IVIM (Intravoxel Incoherent Motion) was able to show decreased perfusion in FGR (Moore et al. 2000b). Subsequently, placental perfusion was measured with different MRI sequences (IVIM and ASL, "arterial spin labeling") and correlated with the resistance (pulsatility index, PI) measured by Doppler sonography in the uterine arteries (Derwig et al. 2013): It was shown that placental perfusion measured by MRI correlates with uterine Doppler and is reduced in pregnancies with small-for-gestational-age infants.

Oxygenation and Placental Metabolism

Intact placental oxygenation is essential for fetal growth and development. Various MRI techniques have been used in recent studies to investigate placental oxygenation and fetal oxygenation (Sørensen et al. 2013; Huen et al. 2013). However, these methods have not yet progressed beyond the research stage.

MRI techniques are also used to study placental metabolism (Denison et al. 2012). Further studies must show whether this will be of importance in the future.

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Section 9.1

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Disorders of Early Pregnancy and Pregnancy Loss

Stephanie Pildner von Steinburg, Ekkehard Schleußner, Ruben Kuon, Kilian Vomstein, and Bettina Toth

Contents

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10.1 Early Pregnancy and Its Disturbance

Stephanie Pildner von Steinburg and Ekkehard Schleußner

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Clinical, laboratory chemistry and sonographic methods allow early and reliable diagnosis and assessment of early pregnancy. Serial determination of human chorionic gonadotropin (hCG)—taking into account appropriate doubling times—allows differentiation between intact and disturbed implantation. Transvaginal sonography can provide information on the site of implantation, the vitality of the embryo and the presence of a multiple pregnancy. In addition, sonographic assessment allows a largely reliable determination of the gestational age.

The term "pregnancy loss" is understood to stand for a (non-artificial) loss of pregnancy before the child becomes viable. It is of importance to distinguish between sporadic and recurrent pregnancy losses, whereby a recurrent pregnancy loss occurs when there are three or more consecutive miscarriages. In the following, the clinical stages and the various causes of sporadic pregnancy losses will be discussed.

10.1.1 Diagnosis of Early Pregnancy

General Remarks

The absence of regular menstruation is the most common indication that pregnancy has occurred, and a detailed history of the menstrual cycle can support this suspicion.

Subjective complaints of early pregnancy include symptoms such as the unusual rejection of certain stimulants and foods, morning sickness with vomiting, breast tenderness and emotional imbalance. Also very early in pregnancy, symptoms such as increased vaginal discharge and a tendency to constipation and pollakiuria may appear as indications of altered bowel and bladder function. These uncertain signs of pregnancy (which by definition also include absence of menorrhea) can appear in varying degrees.

On gynecological examination, further uncertain indications of pregnancy include loosening and livid discoloration of vulva, introitus, vagina and cervix. The loosening of the uterus and in particular of the lower uterine segment, whereat the fingers of the inner and outer hand can almost touch each other on palpation (Hegar pregnancy sign), has been evaluated in the past as a valuable indication of a possibly existing early pregnancy. Often, the enlarged ovary can be palpated with the corpus luteum graviditatis already in the early stage of pregnancy. A palpable enlargement of the uterus, on the other hand, is found at the earliest from the 7^{th}–8^{th} week of pregnancy, but the interindividual variability is known to be high.

However, the clinical findings provide only limited information about the presence, integrity and regular growth of an intrauterine early pregnancy, which is why the use of laboratory chemistry and sonographic methods appears to be useful in answering these questions.

Laboratory Diagnostics

Human Chorionic Gonadotropin (hCG)

The glycoprotein "human chorionic gonadotropin" (hCG) consists of two subunits (α- and β-chain) and is expressed in trophoblast cells. hCG is detectable in maternal serum at the earliest 8 days after ovulation and shows similarities to the luteinizing hormone (LH) with regard to molecular structure and luteotrophic effect. The different structure of the β-chains allows—with the aid of specific antibodies—the differentiation between (β-)hCG and LH in the common

test systems. When pregnancy occurs, hCG increasingly replaces LH, whereat the maintenance of steroid synthesis in the corpus luteum is in the foreground. Here, a sufficiently high progesterone level in the serum is important for the maintenance of early pregnancy.

In the first 10–12 days of an intact intrauterine singleton pregnancy, the doubling time of hCG in serum is about 1.3 days. With increasing gestational age and/or higher hCG levels, the doubling time is prolonged—for example, it is reported to be 3 days at levels between 1200 and 6000 mIE/ml. At this point, however, it should be emphasized that lower initial values and/or a prolonged doubling time of hCG should not be taken as the sole indication of extrauterine pregnancy or disturbed intrauterine early pregnancy. Conversely, short doubling times and/or high levels of hCG do not necessarily indicate the presence of trophoblastic disease.

The highest hCG levels with 50,000–100,000 mIE/ml are found in the tenth week of gestation, after which there is a continuous drop to values around 10,000–20,000 mIE/ml up to the 20th week of gestation. These values remain more or less constant until delivery of the child (Speroff et al. 1994).

Additional Factors, Hormones and Screening Tests

Progesterone is secreted in a pulsatile manner, therefore the values fluctuate between two and 40 ng/ml within a very short time. Due to this, they do not play a major role in practice for the assessment of the regular development of a pregnancy; however, values <5 ng/ml are not compatible with a vital pregnancy.

Other placental products (e.g. "pregnancy-associated plasma protein A", PAPP-A, and "placental growth factor", PlGF) have been established in recent years as part of first-trimester diagnostics for risk assessment of chromosomally abnormal pregnancies and for predicting complications in the second half of pregnancy.

Verification of Early Pregnancy by Means of Sonography

In transvaginal sonography—technically much more advantageous due to the proximity of the transducer to the uterus and thus the better image resolution—the gestational sac can be visualized from a size of about 2 mm. Here, however, it is often difficult to distinguish it from a "pseudogestation sac", an accumulation of fluid in the uterine cavity. A gestational sac can be visualized from 32–36 days post-menstruation, i.e. 4 + 5 to 5 + 1 weeks gestation. For this purpose, the diameter of the gestational sac, also called chorionic cavity diameter, should be measured in three dimensions and the mean value used (Fig. 10.1).

In undisturbed early pregnancy, the chorionic cavity diameter grows on average 1.1 mm per day (Fig. 10.2) and a yolk sac can be visualized in the fifth week of gestation. The absence of a yolk sac as well as below-average growth indicate an early disturbance. If both structures are detected in the uterine cavity, the intrauterine presence of an embryo is confirmed and miscarriages occur in only 11% of cases (Jauniaux et al. 2005).

Embryonic structures can be visualized at the earliest from 5 + 0 weeks gestation with a crown-rump length (CRL) of 1–2 mm and a gestational sac of 5–12 mm in size (Fig. 10.3). With corresponding growth curves, the gestational age can be inferred from the CRL with an accuracy of about 4 days if the gravidity is intact. Only if there is a discrepancy of at least 1 week between the sonographic and calculated gestational age when the CRL is measured twice at intervals of 10 days should the expected date of delivery (EDD) be corrected.

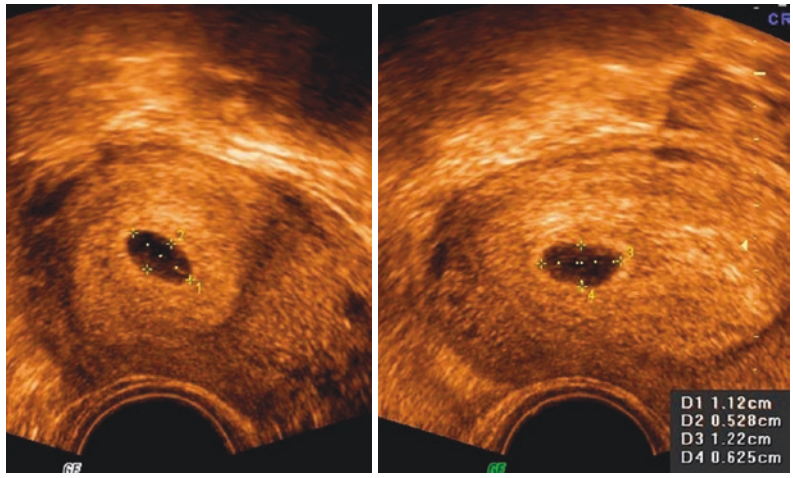


Fig. 10.1 Intrauterine early pregnancy fifth week of gestation still without embryonic structure

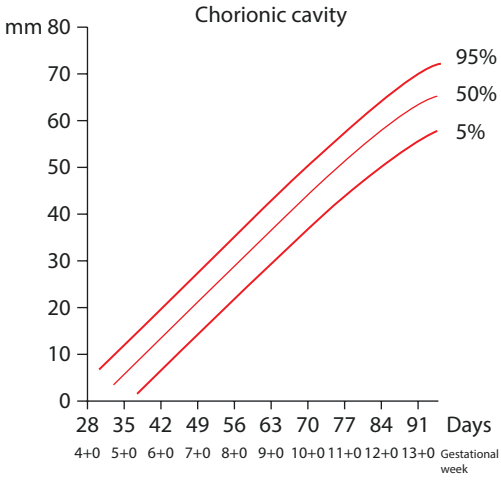


Fig. 10.2 Average growth dynamics of the chorionic cavity diameter. (Modified according to Rempen 1991)

The still to be expected frequency of pregnancy losses decreases with an increase in CRL and amounts to 7.3% from 5 mm, 3.3% from 6–10 mm and only 0.5% above 10 mm (Jauniaux et al. 2005).

An intact early pregnancy is only confirmed by evidence of embryonic cardiac action.

Since the heart does not begin to pulsate until the 23rd embryonic day post conception, it cannot be visualized on ultrasound before 5 + 2 weeks gestation. At the latest from the seventh week of gestation, corresponding to a CRL of 6–9 mm, it must be possible to show a clear cardiac action. In the course of early pregnancy, the heart rate initially increases to 180 beats per minute (bpm) up to the ninth/tenth week of gestation, and then falls to about 140 bpm at the end of the first trimester.

In early ultrasound, the placenta cannot be localized because physiologically it lines the amniotic cavity in a circular fashion as a chorion rich in echoes (Figs. 10.1 and 10.3). It is not until the end of the first trimester that the majority of the chorion regresses and forms the chorion laeve, and then later fuses with the amniotic membrane to form the fetal membranes. In the area of the umbilical cord insertion, the fetal vessels will sprout into the developing placenta and then form the differentiated placenta in this area, which can then also be delineated sonographically.

At this early stage, on the other hand, the detection of a multiple pregnancy is unproblematic. The detection of chorionicity and

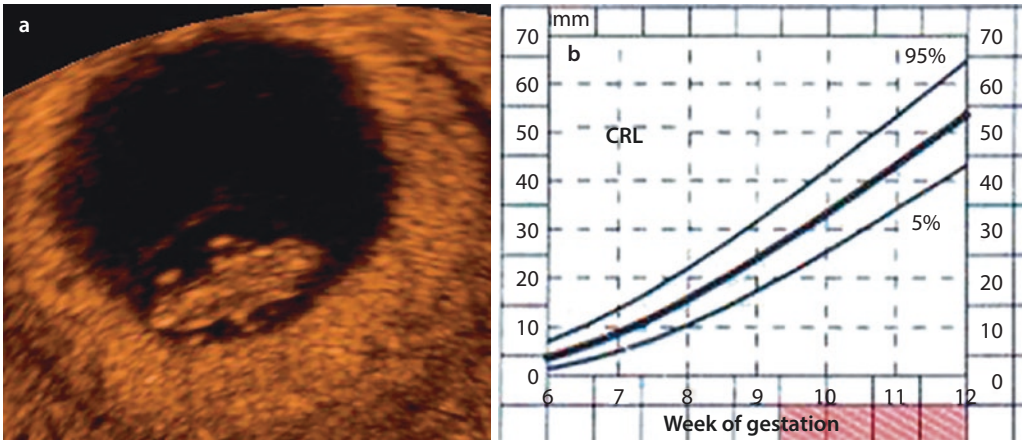


Fig. 10.3 **a** Embryo in the eighth week of gestation, CRL 29 mm, arm and leg buds can be displayed on both sides. **b** Growth curves of the crown-rump length from the German maternity record

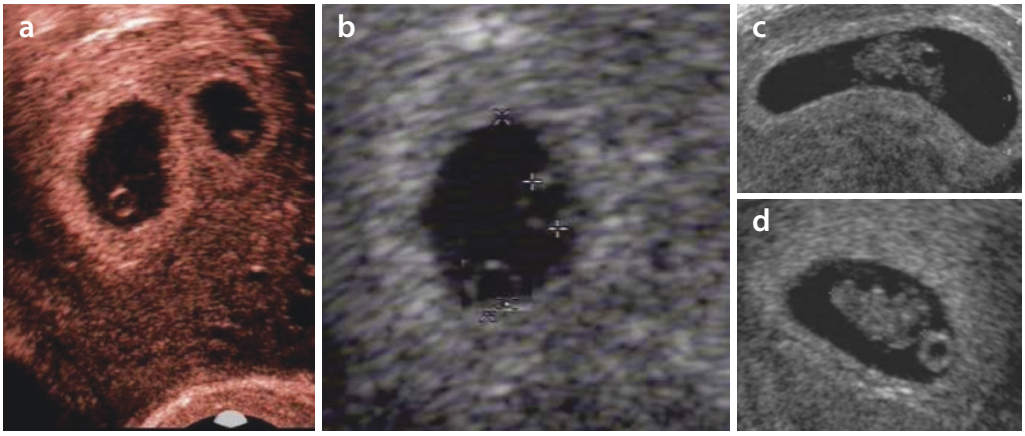


Fig. 10.4 **a**–**c** Differentiation of chorionicity and amnionicity in first trimester multiples. **a** Dichorial-diamnial (2 chorionic sacs, 2 yolk sacs, 2 embryos); **b** monochorial-diamnial (1 chorionic sac, 2 yolk sacs, 2 embryos); **c** monochorial-monoamnial (1 chorionic sac, 1 yolk sac, 2 embryos); **d** normal singleton pregnancy

amnioticity is also much easier than in later weeks of pregnancy, but of crucial importance for the management of possible complications of multiple pregnancy. This is why this must also be documented in the maternity record at the first ultrasound screening.

Sonographic indicators of multiple pregnancy are the number of chorionic cavities, yolk sacs, and embryos (Fig. 10.4).

The lambda sign, which occurs in a dichorial situation and represents placental tissue between the two amniotic membranes, also serves to differentiate mono- and dichorial twin pregnancies (Fig. 10.5).

Already in the first trimester of pregnancy, there are indications for the presence of fetal diseases (conspicuous body contour with regard to neck and abdominal wall, abnormal heart rate, abnormal organ structures, abnormalities of the umbilical cord and/or placenta, conspicuous growth curve, etc.), which can be detected in the first trimester screening in the sense of an early diagnosis of malformation (Merz et al. 2004).

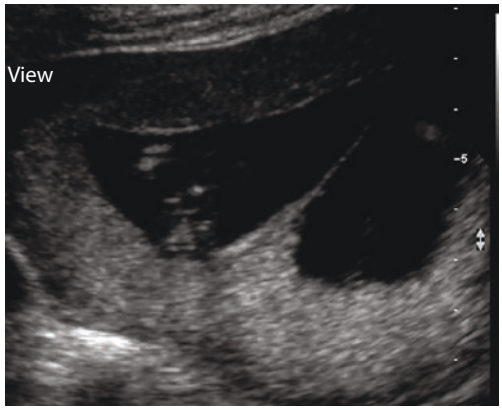


Fig. 10.5 Lambda sign in dichorial-diamnial twins

10.1.2 Pregnancy Loss

Definition and Epidemiology

The term "pregnancy loss" refers to a (non-artificial) loss of pregnancy before the child is viable, defined by a birth weight of <500 g (WHO definition).

The rate of clinical pregnancy losses after the absence of menorrhea, in relation to the total number of all pregnancies detected, is on average between 11% and 15%. Up to four times more embryos are likely to abort unnoticed in the short period between implantation and menstruation, and a further 15% or so before implantation, which implies that the combined rate of preclinical (before the absence of menstruation) and clinical pregnancy losses is likely to be >50%.

Sporadic miscarriages are distinguished from recurrent miscarriages, which are defined as three or more consecutive miscarriages. From a clinical point of view, a division into early pregnancy losses up to the 12th–14th week of gestation and late pregnancy losses from the 14th week of gestation onwards makes sense. However, with regard to the etiology, except for endocrine causes and cervical insufficiency, the transition between early and late pregnancy losses seems to be smooth. For this reason, the etiological factors that can lead to miscarriages in the first and second trimesters are dealt with together in the corresponding sections of the chapter.

So far, little attention has been paid to the clinical significance that the history of miscarriages has for the outcome of an existing pregnancy: In addition to the increasing probability of another miscarriage, the risk of a (very early) preterm birth, caused by premature rupture of the membranes or preterm labor, doubles already after the first miscarriage (Buchmayer et al. 2004).

Clinical Stages of the Abortion Process

Clinically, a **threatened abortion** (abortus imminens) is accompanied by vaginal bleeding with or without uterine contractions, usually without opening of the external cervical os. Sonographically, a vital embryo/fetus, a preserved uterine cervix and a closed internal cervical os are seen. The detection of a possibly present perichorial hematoma allows the differential diagnosis of a portioectopic hemorrhage or other causes of bleeding. Laboratory chemistry shows regular doubling times of hCG up to the tenth week of gestation.

Although generally recommended, the clear benefit of physical rest or bed rest with regard to pregnancy outcome in the case of threatened miscarriage in the first trimester has not yet been proven (Aleman et al. 2010). There are also no satisfactory controlled studies on drug treatment with progestogens and/or hCG. However, there seems to be evidence that the use of progesterone orally (50–100 mg per day), dydrogesterone (10 mg two times per day) or hCG (5000 I.U. weekly to three times 9000 I.U. weekly) can reduce the rate of miscarriages later in life (meta-analyses in Devaseelan et al. 2010; Wahabi et al. 2011; Carp 2012, 2015). However, the high rate of chromosomal anomalies in sporadic miscarriages (see below) must be taken into account, and

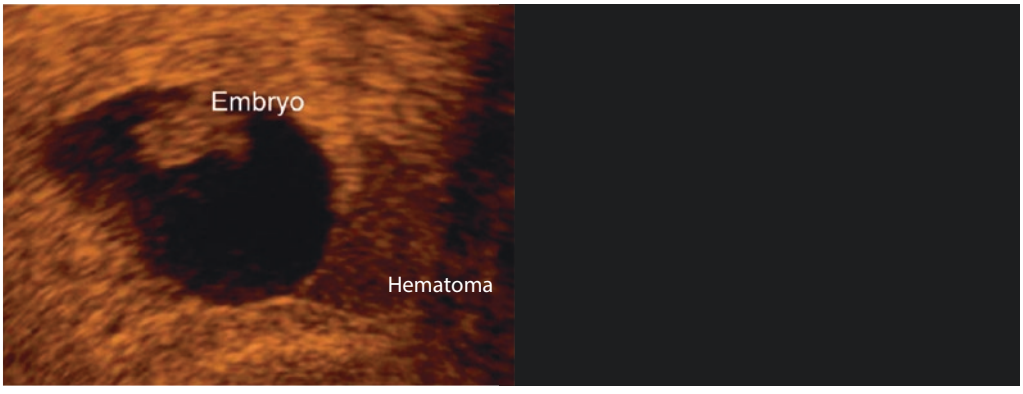
none of the therapeutic measures indicated can change the outcome of the pregnancy.

If cardiac actions are present in the first trimester when abortion is threatened, a favorable course of pregnancy can be expected in about 90% of cases. This is of high practical importance for the reassurance of the affected patient.

In **incipient abortion** (abortus incipiens) there is a softening and shortening of the uterine cervix with dilatation of the cervical canal under labor-like uterine contractions. In this situation, sonography allows confirmation of the clinical findings at the cervix—in addition, a deformed chorionic cavity with a non-vital embryo/fetus and a pronounced perichorial hematoma are often found (◘ Fig. 10.6).

If partial or complete spontaneous expulsion of the embryo/fetus and the placenta has already occurred, an **incomplete or complete abortion** is present. On clinical examination, the uterus is often smaller than is appropriate for the gestational age, and a gaping cervical canal is found, although this may have reformed in the case of a complete abortion. Sonography cannot detect an intrauterine chorionic cavity with a vital embryo. Difficulties are often caused by the sonographic differentiation between placental remnants remaining in utero and blood clots, each of which may appear as irregular echo-dense structures. Since both clinically and sonographically a differentiation between a complete and incomplete abortion is not possible with certainty, a vacuum curettage can be offered—especially in cases of persistent bleeding.

In a **missed abortion** there is no spontaneous abortion despite the fact that the embryo/fetus has died or is no longer developing. Clinically, the uterus is smaller than corresponding to the period of amenorrhea, the cervix appears coarse, and the cervical canal is closed. In the context of hCG determinations, it is not a possibly lowered baseline value that is of importance, but the absence or delayed doubling of the values within a period of time. On ultrasound, a non-vital embryo/fetus is found passively following the movements of the amniotic fluid in the course of a push palpation of the uterus. From a CRL of 7 mm, cardiac actions must be sonographically detectable. Biometry often shows an embryo that is too small in relation to the chorionic cavity diameter and to the calculated gestational age. If no embryonic parts can be visualized with a mean chorionic diameter > 25 mm, a "blighted ovum" must be assumed. In cases of prolonged missed abortion, severe coagulation disorders with the development of disseminated intravascular coagulopathy (DIC) have been reported in



◘ **Fig. 10.6** Amorphous embryo and perichorial hematoma in missed abortion

individual cases. However, this is a very rare complication.

The rule used to be that curettage was necessary for retained pregnancy material after abortion to prevent infection. However, a three-arm study comparing expectative with medical (vaginal misoprostol) and surgical procedures in 1200 women with missed abortion or incomplete abortion before 13 completed weeks of gestation (Trinder et al. 2006) showed no differences in (low) infection rates between the two collectives. The rates of post curettage (indicated because of heavy bleeding or sonographic suspicion of retention after 14 days) were 44% vs. 13% vs. 5%. For incomplete abortion, prospective management showed a success rate of 75%, but with a rate of unplanned inpatient admissions of 29%. For missed abortion, the proportion of women requiring surgical intervention was lowest in the drug treatment arm at 38%, but with longer hospital stays.

A recent Cochrane analysis (Kim et al. 2017) also concluded that medication or wait-and-see is an acceptable alternative to curettage based on outcome, but the data for fertility preservation of the different methods is not yet robust.

If the cervix is closed, prostaglandins should be used preoperatively to soften the cervix in order to prevent surgical complications and subsequent cervical insufficiencies. Gemeprost or misoprostol (off-label use!) are available for this purpose.

In surgical intervention, suction curettage should be preferred because of the lower complication rates. If increased bleeding occurs during/after surgical uterine evacuation, drug-based augmentation of uterine contractions must be performed as in the case of atonic postpartum hemorrhage.

If the size of the gestational sac corresponds to that of the second trimester, a medically assisted spontaneous expulsion should first be attempted. Here, a subsequent curettage is usually necessary to remove any remaining placental remnants. Various prostaglandins (Gemeprost, Sulproston, Dinoproston, Minprostin and Misoprostol) as well as Oxytocin are used depending on the maturity of the cervix.

With regard to perioperative antibiotic prophylaxis, various substances, dosages and administration methods have been tested: The optimal regimen could not yet be determined on an evidence level due to the many different therapeutic approaches, but tetracyclines, ceftriaxone and metronidazole can reduce post-interventional infection rates with manageable side effects (overview in Morrill et al. 2013).

The presence of an **infected abortion** is indicated by an increase in temperature > 38 °C, a leukocytosis of ≥12,000 and a significant increase in the erythrocyte sedimentation rate and C-reactive protein (CRP) level. In most cases, the clinical stage of an incipient or incomplete abortion is present, and in this situation, a previous attempted abortion must also be considered. In the initial stage, the infection affects the uterine cavity, but in the further course it may spread to the parametria with adnexa, to the peritoneum and hematogenously in the sense of a septic abortion. A frequently underestimated early symptom of an incipient septic event is persistent hypotension with tachycardia, which may initially be misinterpreted as vegetative dystonia or as a consequence of volume deficiency. In the further course, septic shock may develop with the cardinal symptoms of circulatory and renal failure as well as manifest disseminated intravascular coagulopathy (DIC) with a mortality rate of about 20% (Finkielman et al. 2004).

The specific therapy of an infected or septic abortion should therefore be started as early as possible and comprises, after obtaining bacterial cultures,

- high-dose intravenous antibiotics (e.g. clindamycin + gentamycin ± ampicillin or ampicillin + gentamycin + metronidazole or levofloxacin + metronidazole or

imipenem or single substances with a similar spectrum),
- adequate volume substitution and
- the control of coagulation parameters, in order to be able to detect an incipient DIC as early as possible.

In the case of a uterine size >14 weeks of gestation, a drug-assisted expulsion can be aimed at the same time. Before the 14th week of gestation, surgical emptying or palpation of the uterus can be planned immediately after the start of adequately dosed intravenous antibiotic therapy, as the risk of further spread of septicemia in connection with the surgical intervention is low. Due to the antibiotics available nowadays, this procedure has proven to be more effective than waiting too long and the risk of developing endotoxin shock.

Causes of Pregnancy Loss

Chromosomal Abnormalities in Pregnancy Tissue

Numerical chromosomal abnormalities are detected in 50–70% of all sporadic miscarriages, in 90% of early miscarriages, in 50% of miscarriages in the 8th–11th week of gestation, 30% in the 16th–19th week of gestation and 6–12% beyond the 20th week of gestation (overview in Warren and Silver 2008). The most common of these aneuploidies are autosomal trisomies (60%) (most commonly trisomy 16, 20–30% of trisomies) and monosomy X (20%) and polyploidies (20%) followed by structural anomalies (Warren and Silver 2008). Mosaics confined to the chorion or placenta are also found more frequently in sporadic pregnancy losses (Kalousek et al. 1992). In contrast, abnormalities in the embryonic/fetal karyotype are not among the common causes of recurrent pregnancy losses. On the contrary, a normal set of chromosomes is often found in the pregnancy tissue of couples with recurrent pregnancy losses (Sullivan et al. 2004).

Genetic Causes in the Parents

Genetic causes of recurrent pregnancy losses include chromosomal abnormalities of one parent, molecular defects, and multifactorial syndromes.

Mutations and genetic defects may be responsible for a significant proportion of euploid pregnancy losses. However, the molecular genetic techniques to detect more of such associations have only recently become available, and systematic insights into the role of mutations in causing recurrent pregnancy losses are still lacking.

Uterine Anomalies

Congenital Uterine Anomalies

There is no reliable information on the general incidence of congenital uterine anomalies. In women with recurrent pregnancy loss, uterine malformations are reported in 10–30% of cases, while the risk of pregnancy loss is likely to depend primarily on the type of anomaly present and its severity. In some cases, the literature contains very different data on the rate of pregnancy losses in the various uterine malformations (Table 10.1).

In general, the increased risk of obstetric complications in all malformations of the Müllerian ducts should be mentioned, such as prematurity, fetal growth restriction, anomalies of the position and pole onfigurations and uterine ruptures after surgical correction.

Table 10.1 Risk of pregnancy loss depending on the type of uterine malformation. (Overview in Grimbizis et al. 2001)

Risk of pregnancy loss	Frequency (%)
Uterus septus/subseptus	44.3
Uterus unicornis	36.5
Uterus bicornis	36
Uterus didelphys	32.2
Uterus arcuatus	25.7

Acquired Uterine Anomalies

Intrauterine synechiae after endometritis and intrauterine surgery and, more recently, after transcavitary compression sutures, described in the context of atony treatment after a previous delivery, are also considered a risk factor for early and late pregnancy losses as well as for placental disorders (Poujade et al. 2011). The incidence of intrauterine synechiae and their extent increases with the number of previous losses and intrauterine interventions: for example, the incidence of such adhesions is 14–16% after two, but 32% after three or more early losses (Friedler et al. 1993).

Even large myoma often remain asymptomatic during pregnancy. However, the presence of submucosal myoma is associated with an increased risk of preterm and late losses and other pregnancy complications (preterm births, premature placental abruption).

The importance of cervical insufficiency in triggering pregnancy losses—mostly in the second trimester—has probably been overestimated in the past and is likely to be around 1% in unselected pregnant women, but around 13% in pregnant women with a history of recurrent pregnancy losses (Stray-Pedersen and Stray-Pedersen 1984). The recurrence risk of late pregnancy loss or extreme preterm birth in cervical insufficiency is 28% (Sneider et al. 2016). The clinical presentation of cervical insufficiency may be gradual, painless dilatation of the cervix, bulging of the amnion, or premature rupture of the membranes with a uterus without labor. However, any of these symptoms may also occur during the course of a pregnancy loss of a different cause and therefore does not prove the causality of cervical insufficiency for the loss of pregnancy.

Infections

A number of bacterial, parasitic and viral infections are causally associated with sporadic pregnancy loss. The associations of syphilis, listeriosis and toxoplasmosis as well as various viral infections with pregnancy outcome are well known. Lyme disease is also still discussed as a cause of miscarriages and malformations.

Bacterial vaginosis is a recognized risk factor for late pregnancy losses, preterm births and premature rupture of membranes. However, an association with pregnancy losses during the first trimester was not confirmed in a meta-analysis (van Oostrum et al. 2013), while there was an association with loss of preclinical pregnancies. In addition, when assessing the causality of bacterial vaginosis for the occurrence of pregnancy losses, the coincidence with other genital infections (chlamydia, mycoplasma) that potentially cause miscarriages must also be taken into account.

Stimulants and Pollutants

Nicotine and/or increased caffeine consumption seem to be associated with an increased risk of miscarriage—however, these associations have not been proven in all studies. Significantly increased numbers of miscarriages, on the other hand, are found in women with chronic alcohol, opiate and cocaine abuse during pregnancy.

Occupational exposure to cytostatic drugs and anesthetic gases has been recorded to increase the rate of pregnancy losses among medical personnel. Workers in certain sectors of the metal industry, in chemical or pharmaceutical plants, in dry cleaning and women handling organic solvents or paints also appear to have an increased risk of miscarriage, although the differences with the miscarriage rates of unexposed women appear to be significant only for certain substances or combinations.

In view of the large number of substances in question and the variable duration and intensity of exposure, the partly contradictory findings on the role of pollutants at the workplace and in the domestic environment in triggering pregnancy losses are not surprising, especially since knowledge of the

fertility-inhibiting potential of individual substances and their additive effects is still fragmentary.

Endocrine Causes

Obesity has generally been shown to be a risk factor for miscarriage (Boots and Stephenson 2011), not only in the field of assisted reproduction or in the presence of polycystic ovary syndrome (PCOS). However, data demonstrating that normalizing body weight—in addition to regulating the menstrual cycle—can also reduce the risk of miscarriage are lacking to date (Best et al. 2017). A prediabetic metabolic state, recognizable by an elevated HOMA index (ratio of fasting blood glucose to insulin) or a pathological oral glucose tolerance test, can also cause recurrent pregnancy losses. Preconceptional therapy with metformin can be promising in this case. In the course of pregnancy, insulin therapy is often necessary in cases of gestational diabetes (Zolghadri et al. 2008).

Diabetic pregnant women whose glucose levels are well controlled are not at significantly higher risk of miscarriage than pregnant women without diabetes mellitus. On the other hand, the likelihood of miscarriage and malformations of the child is clearly increased in pregnant diabetics with poor metabolic control during the first trimester, i.e. with high levels of glucose and glycosylated hemoglobin (Deutsche Diabetes-Gesellschaft 2014).

Hypo- or hyperthyroidism are often mentioned as possible causes of sporadic or recurrent pregnancy losses, but there are also reports to the contrary in the literature. Although a thyroid dysfunction is to be expected in only up to 2% of women with recurrent pregnancy loss, an examination of the thyroid function in these patients seems to be justified with regard to the easy correctability of these dysfunctions and also because of a possible worsening during pregnancy. The lower normal range should be considered as the target TSH level, even though only small improvements in miscarriage rates have been shown in the literature (Reid et al. 2010). An association of pregnancy loss with the presence of thyroperoxidase (TPO) antibodies has been shown, but it is unclear whether therapeutic measures other than correction of thyroid function are required (Prummel and Wiersinga 2004). A reduction of the postpartum thyroiditis rate could be achieved by the additional use of selenium (Reid et al. 2010).

Psychosocial Factors

The psychological trauma as a consequence of one or even several pregnancy losses is widely underestimated. The awareness of having lost a pregnancy is intensified by the possibilities of early sonographic diagnosis. After experiencing multiple pregnancy losses, the fear of recurrent pregnancy losses is all too understandable, which is why a high incidence of reactive depression and anxiety is found in couples with recurrent pregnancy loss. Although psychological factors are unlikely to be the cause of repeated pregnancy loss, neglecting these aspects is potentially detrimental. Several studies have demonstrated a high rate (75%) of successful pregnancies in patients with recurrent pregnancy loss solely through "tender loving care" in conjunction with short-term clinical and sonographic monitoring (Stray-Pedersen and Stray-Pedersen 1984; Rai et al. 1996), although the high spontaneous success rate must be taken into account when evaluating these measures.

10.2 Recurrent Pregnancy Loss

Ruben KuonKilian Vomstein, and Bettina Toth

10.2.1 Introduction

While about 30% of all women experience a spontaneous pregnancy loss in their lifetime, the incidence of recurrent (habitual) sponta-

neous pregnancy loss (RPL) is 1–3% depending on the definition used. The WHO defines the presence of RPL after three consecutive miscarriages before the 20th week of gestation, the American Society for Reproductive Medicine already speaks of RPL after two consecutive miscarriages.

Established risk factors include endocrine, anatomical, infectiological, genetic, psychological, hemostaseological and immunological factors (Fig. 10.7). After standardized diagnostics, an explanatory cause can be identified in about 50% of affected women; the other half of RPL remains unclear, which is why the establishment of new diagnostic and therapeutic approaches is urgently needed.

In the following section, we will first discuss the individual established risk factors. We then focus on potential new risk factors such as chronic endometritis (CE) and the presence of peripheral and uterine natural killer cells (pNK and uNK cells) in patients with RPL.

10.2.2 Established Risk Factors

Endocrine Dysfunctions

Endocrine causes of RPL include luteal phase insufficiency, thyroid dysfunction, metabolic syndrome including obesity, and PCO syndrome (PCOS) (Fig. 10.8).

The diagnosis of luteal phase insufficiency includes determining the length of the menstrual cycle, measuring the progesterone level in the luteal phase and, if necessary, performing an endometrial biopsy to detect secretory transformation of the endometrium. To date, there are no studies that have demonstrated a clear association between the occurrence of RPL and the diagnosis of luteal phase insufficiency.

Thyroid dysfunctions include both manifest hyperthyroidism and manifest hypothyroidism, both of which are associated with the occurrence of miscarriages (Anselmo et al. 2004). At present, however, the data are unclear as to what extent latent

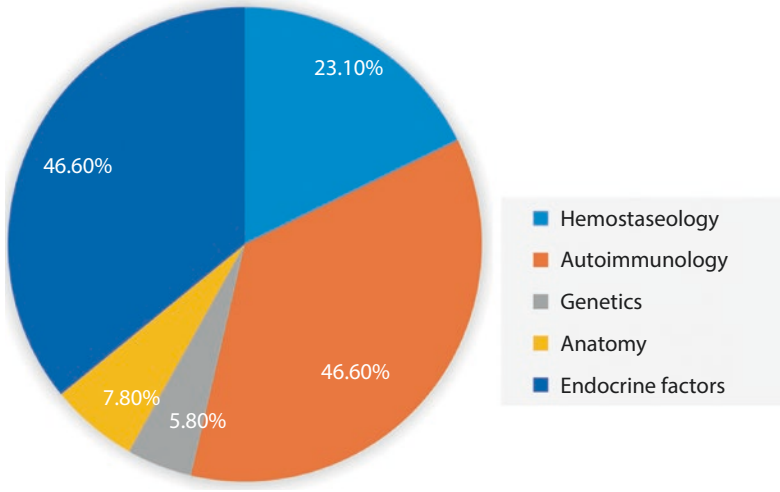


Fig. 10.7 Incidence of individual established risk factors in RPL patients (own previously unpublished data). The respective risk factors include: Endocrine factors: thyroid disease (hypo−/hyperthyroidism, anti-TPO antibodies) and luteal phase insufficiency; Autoimmunology: antinuclear antibodies (ANA titer >1:160), anti-cardiolipin antibodies (ACL IgG/IgM), anti-ß2-glycoprotein IgG/IgM, lupus anticoagulant; Hemostaseology: Factor V Leiden, prothrombin or methyltetrahydrofolate reductase (MTHFR) mutation, protein C/S deficiency, antithrombin deficiency; Genetics: parenteral chromosomal disorders; Anatomy: uterine septum

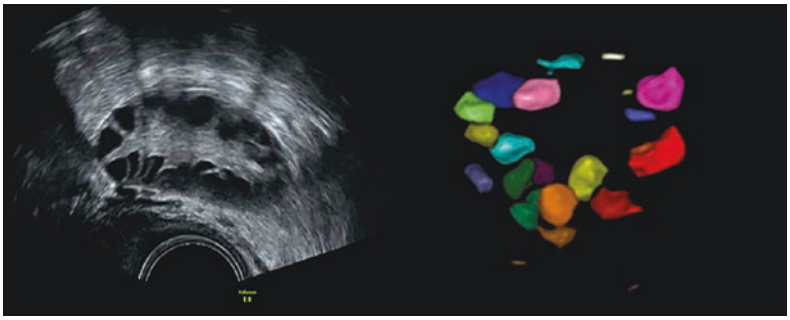


Fig. 10.8 PCO-typical ovaries in transvaginal ultrasound. The classic pearl string-like arrangement of the follicles is depicted. (With kind permission of Prof. Dr. Brezinka, MUI Innsbruck)

hypothyroidism (elevation of TSH concentrations in the presence of normal thyroid hormone concentrations) is also associated with (recurrent) miscarriages. In principle, the Endocrine Society recommends an upper TSH value of 2.5 mU/l (Abalovich et al. 2007), which should already be reached pre-conceptually.

The pathophysiological significance of the metabolic syndrome including obesity and PCOS for the occurrence of RPL is often correlated as there is overlap.

Even though the data from international studies are inconsistent, the BMI should be determined in patients with RPL and a metabolic syndrome should be investigated for, if the BMI is ≥30 kg/m^2. Prior to a new pregnancy, weight reduction should already be attempted.

Anatomical Malformations

Anatomical causes include both congenital (such as uterine malformation or other urogenital malformations) and acquired (such as intrauterine adhesions, polyps, myoma) malformations.

The incidence of uterine malformations in patients with RPL varies between 3–25% in studies (Salim et al. 2003; Sugiura-Ogasawara et al. 2011), with uterus septus being the most common.

Pathophysiologically, it is assumed that the septum leads to a disturbance of vascularization in the course of placental development (Raga et al. 2009). Therefore, surgical hysteroscopy with septum resection is recommended prior to the onset of a new pregnancy. Similarly, there is evidence that a so-called uterus unicornis is associated with the occurrence of RPL or late pregnancy losses or preterm births (Ozgur et al. 2017), however, therapeutic approaches are lacking.

The diagnosis of congenital or acquired uterine malformations is performed using (3D) vaginal sonography or diagnostic hysteroscopy.

Acquired anatomical disorders include intrauterine adhesions, which may occur mainly after dilation and curettage or infection, as well as myoma and polyps. Intrauterine adhesions can be detected by diagnostic hysteroscopy and should be removed.

Depending on their location, myoma/fibroids are classified as submucous, intramural and subserous (see also FIGO classification). Submucosal and large intramural myoma, which compress the uterine cavity, are associated with the occurrence of RPL. However, data is sparse and inconsistent. Nevertheless, in the presence of submucous or intramural myoma compressing the uterine cavity, a pre-conceptional hysteroscopic or laparoscopic enucleation can be performed.

The same applies to intracavitary polyps. As in cases of myoma, there is no international consensus on the size or number of

polyps that are relevant for the occurrence of RPL and therefore have to be removed preconceptionally. Nevertheless, the removal of polyps with histological examination is recommended from a size of 15 mm to exclude a (rare) malignant degeneration.

Infections

Especially in the presence of recurrent late pregnancy losses with premature rupture of the membranes, premature labor and opening of the cervix as well as amniotic infection syndrome, vaginal infections should be excluded in the patient. In the course of a gynecological examination, both a smear from the vagina and a bacteriological sample should be taken. In the event of an infection, antibiotic treatment should be administered as proven in the antibiogram.

For prophylactic purposes, vaginal suppositories containing Döderlein bacteria can be used before pregnancy.

Chromosomal Disorders

After the sperm has fertilized the oocyte, a number of developmental steps are necessary for proper embryonic development to occur.

These developmental steps are highly error-prone, which leads to frequent (unnoticed) miscarriages or failure of embryo implantion in a woman's lifetime (Laurino et al. 2005). Furthermore, the probability of embryonic or fetal chromosomal maldistribution shows a strong correlation with maternal age, and hereditary genetic disorders may also be present.

Concerning chromosomal aberrations in miscarriage tissue, trisomy 16 is identified most prevalently, however, trisomy 22, triploidy and monosomy X (Turner syndrome) are also found frequently.

Only about 4–5% of RPL couples are diagnosed with a (balanced) chromosomal change in at least one of the partners. Not all international guidelines recommend a standardized chromosome analysis in the affected couples (De Braekeleer and Dao 1990). The guidelines of the German, Austrian and Swiss Society of Gynecology and Obstetrics (DGGG, OEGGG, SGGG) for the diagnosis and treatment of RPL currently recommend a human karyotyping of the affected couple or the product of conception.

In principle, prior to any genetic diagnosis, an information on the planned genetic diagnostics should be performed by a qualified physician, including the written consent of the couple or patient concerned, in accordance with the genetic diagnostics law of the respective country.

If one of the partners of the affected couple is diagnosed with a balanced chromosomal abnormality, the risk of a pregnancy loss as well as the risk of giving birth to a child with a chromosomal abnormality depends on the chromosomes affected.

There is no causal therapy for the treatment of maternal or paternal chromosomal abberations or for the prevention of unbalanced chromosomal rearrangements. However, pre-implantation genetic diagnosis (PGD) can be used to perform a genetic diagnosis of the embryo before transfer.

In PGD, a distinction is made between the examination of already known genetic diseases (e.g. monogenic disease or balanced chromosomal aberration) and the screening for embryonic chromosomal aberrations.

Internationally, the terms "preimplantation genetic testing" (PGT) and "preimplantation genetic diagnosis" (PGD) are increasingly used. Sole screening, on the other hand, is referred to as "preimplantation genetic screening" (PGS). In principle, PGD is performed on trophectoderm cells; the examination of blastomeres has lost priority.

The prerequisite for performing PGD in Germany is regulated in the Embryo Protection Act and is only permitted at PGD centers approved for this purpose. In addition, a positive vote by an ethics committee must be obtained before any PGD is performed in Germany.

In contrast, no ethical vote is required in advance for polar body diagnostics (PBD). However, PBD only permits an inference to maternal chromosomal maldistributions, the paternal side remains unclear.

In the case of PGD (including PBD), the affected patient undergoes hormonal stimulation as part of assisted reproductive therapy (ART), which is costly and time-consuming and may involve risks of side effects.

A PGS enables the selection of genetically healthy embryos. However, contrary to expectations, no improvement in the live birth rate in patients with RPL and PGS has been observed in international studies to date, so that the DGGG/OEGGG/SGGG guideline does not currently recommend the performance of a PGS in RPL patients.

Psychological Factors

Experiencing RPL is a perturbing experience for the couple affected and can lead to severe traumatization. Particularly in the case of pre-existing psychiatric illnesses, close supervision together with a psychologist or psychiatrist should be initiated preconceptually.

Hemostasiological Factors

The clarification of hereditary thrombophilias in patients with RPL is currently the subject of international controversy.

Classical hereditary thrombophilias, which have been investigated in numerous international studies in RPL patients, include mutations in the factor V Leiden (FVL; c.1601G > A in F5, rs6025) or prothrombin gene (G20210A) (PT; c.*97G > A in F2, rs1799963) as well as polymorphisms in the methylenetetrafolate reductase gene (MTHFR C677T or c.665C > T). Furthermore, a deficiency of antithrombin, protein C, protein S, protein Z or factor XII as well as an increased concentration of factor VIII or lipoprotein (a) (Toth et al. 2008) are associated with RPL. Pathophysiological considerations assume that hereditary thrombophilia has an additive effect on the already physiologically present increased procoagulatory systems in pregnancy. As a consequence, microthrombi could form in the placental bed, which in turn cause a reduced blood flow to the placenta and thus reduce supply of the embryo or fetus.

Up to 15% of the Caucasian population exhibit one of the thrombophilic parameters mentioned (Roberts et al. 2009), so that it is evolutionarily questionable why such thrombophilias have been passed on over such a long period of time. One possible explanation could be the positive effect of a procoagulant tendency: It is possible that the patient suffers less blood loss peripartum, which may have been an evolutionary advantage until just a few years ago.

Due to the inconsistent data situation, international guidelines do not recommend screening for maternal hereditary thrombophilia outside of studies. However, thrombophilia screening should be performed if risk factors are present (family history of thrombosis, own history of thrombosis).

The administration of low-molecular-weight heparins (LMWH) in patients with RPL and the presence of a hereditary thrombophilia is recommended in international guidelines, but only in risk constellations and primarily for the reason of maternal thrombosis prophylaxis, but not for the prevention of a new pregnancy loss. This recommendation is mainly based on the results of numerous international studies, which could not prove a benefit with regard to the live birth rate after the administration of LMWH, neither in the presence of thrombophilia nor in so-called idiopathic RPL (without identification of an established risk factor).

In special risk constellations such as the presence of an antithrombin deficiency, a homozygous FVL mutation or a combined heterozygous FVL and PT mutation, however, interdisciplinary care should be provided together with hemostaseologists, obstetricians and, if necessary, neonatologists in addition to (therapeutic) heparinisation of the pregnant woman.

Immunological Factors

Immunological risk factors for RPL include a variety of allo- and autoimmunological factors. However, only the antiphospholipid syndrome (APS) is established as an immunological risk factor for RPL in international guidelines.

By definition (see following overview), laboratory criteria must be fulfilled in addition to clinical criteria for the diagnosis of APS. APS is present in about 2–15% of patients with RPL (Branch et al. 2010). In order to diagnose an APS, laboratory testing of antiphospholipid antibodies in medium to high range (>99th percentile measured in normal subjects) has to be redone 12 weeks after the initial positive blood test (Miyakis et al. 2006a, b).

Diagnostic Criteria for Antiphospholipid Syndrome (Miyakis et al. 2006a, b)

Clinical Criteria:

- ≥1 venous or arterial thrombosis
- One or two unexplained pregnancy losses in morphologically normal fetuses >10th week of gestation
- ≥3 pregnancy losses <10th week of gestation
- ≥1 late pregnancy loss or preterm birth <34th week of gestation due to placental insufficiency or preeclampsia

Laboratory criteria (detection twice at 12-week intervals):

- Anti-cardiolipin antibodies (IgM, IgG), medium to high titers
- Anti-ß2-glycoprotein-1 antibodies (IgM, IgG), high titers
- Lupus Anticoagulant

The individual clinical and laboratory criteria can occur together, but also individually. By definition, however, at least one clinical and one laboratory criterion must be fulfilled in order to establish the diagnosis of an antiphospholipid syndrome.

In addition to the "classic" APS, there are indications that a so-called non-criteria APS can also occur in RPL, in particular if the patients suffer from skin changes (livedo reticularis, ulcerations), a renal microangiopathy, neurological or cardiac disorders and the diagnostic criteria of the classic APS are not or only partially fulfilled (e.g. APS antibody titer in the low range or condition after two pregnancy losses) (Miyakis et al. 2006a, b).

The administration of aspirin (50–100 mg/d) and LMWH in prophylactic doses in patients with RPL and the presence of APS is consistently recommended (Committee on Practice Bulletins-Obstetrics and Gynecologists 2012; Empson et al. 2002, 2005; Mak et al. 2010; Ziakas et al. 2010). In this context, aspirin can be given preconceptionally or from positive pregnancy test and should be continued until the 34 + 0th week of gestation (Derksen et al. 2004). LMWH should be started from a positive pregnancy test and treatment should be continued until at least 6 weeks postpartum.

According to current studies, patients with RPL and presence of non-criteria APS should be treated similarly (Alijotas-Reig et al. 2012; Arachchillage et al. 2015; Cohn et al. 2010; Gardiner et al. 2013).

10.2.3 Possible New Risk Factors

Chronic Endometritis

The prevalence of chronic inflammation of the endometrium (chronic endometritis, CE) in women with RPL is inconsistently reported in the literature between 9–57.8% (Bouet et al. 2016; El Hachem et al. 2017; Kitaya 2011; McQueen et al. 2014, 2015; Park et al. 2016; Zolghadri et al. 2011).

In addition to possible geographical differences and heterogeneous inclusion criteria in the individual studies, the range of prevalence can also be explained in particular by different diagnostic examination methods in the studies available to date.

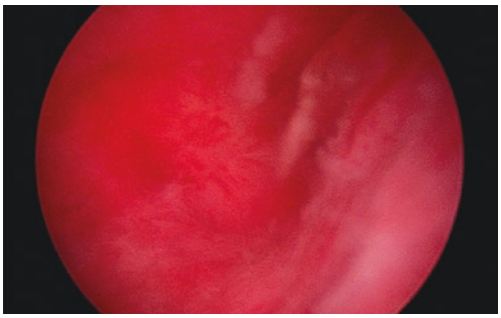
There is still no international consensus regarding the definition of CE or the ideal detection method.

CE is usually asymptomatic or presents with only mild or non-specific symptoms such as chronic lower abdominal pain, dyspareunia, (irregular) vaginal bleeding and persistent vaginal discharge (Greenwood and Moran 1981; Romero et al. 2004). This explains why it remains undetected in many cases (◘ Fig. 10.9).

Several studies point to an infectious etiology of CE (Cicinelli et al. 2014; Park et al. 2016). For example, Cicinelli et al. showed that positive bacterial cultures were present in 75% of cases histologically diagnosed with CE (including Escherichia coli, Enterococcus faecalis, Streptococcus agalacticae in 77.5%, Mycoplasma/Ureaplasma in 25%, and Chlamydiae in 13%) (Cicinelli et al. 2005, 2009, 2014).

CE possibly leads to a restriction of endometrial receptivity via an abnormal infiltration of plasma cells and thus a secretion of IgM, IgG and IgA antibodies (Kitaya et al. 2014). Furthermore, it has been shown that in the case of CE, endometrial gene expression is disturbed with increased transcription of genes that are crucially involved in an immune response to inflammatory stimuli as well as proliferation and apoptosis processes (Di Pietro et al. 2013).

Diagnostic procedures currently used are primarily endometrial biopsy with immunohistochemical workup and diagnostic hysteroscopy.



◘ **Fig. 10.9** Hysteroscopy: chronic endometritis with marked mucosal edema and redness

Immunohistochemical detection of CE is the gold standard and is based on the detection of plasma cells in the endometrium (Chen et al. 2016; Greenwood and Moran 1981; Kannar et al. 2012; McQueen et al. 2014, 2015). Currently, the most sensitive marker for this is immunohistochemical staining with syndecan-1 (CD138) (Bayer-Garner and Korourian 2001; Chen et al. 2016; McQueen et al. 2014; Park et al. 2016). This is a heparan sulfate proteoglycan that is localized on the cell surface and plays a significant role in cell growth and proliferation. Compared to the diagnosis of CE using a simple hematoxylin and eosin (H&E) stain, the use of CD138 has many advantages:

- Reduction of the false negative rate as immunohistochemistry better identifies plasma cells (Bayer-Garner and Korourian 2001; Chen et al. 2016; McQueen et al. 2015);
- Reduction of the false positive rate, as mononuclear cells may be misclassified as plasma cells in H&E staining (Bayer-Garner and Korourian 2001);
- Reduction of the time spent on diagnostics for the pathologist (Bayer-Garner et al. 2004).

A recent study in patients with ≥2 consecutive pregnancy losses before 14 weeks gestation (n = 53) showed a prevalence of CE (defined as detection of ≥5 CD138-positive plasma cells in ten non-overlapping high-power fields [×400]) in 27% of cases (Bouet et al. 2016). Two other studies that used CD138 for immunohistochemical detection indicate a prevalence of CE of 9.3–56% in women with RPL (Kitaya 2011; McQueen et al. 2015).

There is currently no consensus as to whether the detection of even one plasma cell is sufficient for the diagnosis of CE (Johnston-MacAnanny et al. 2010; Kasius et al. 2011; McQueen et al. 2015), or whether a certain number must be present (Bouet et al. 2016) (◘ Fig. 10.10). Similarly, the

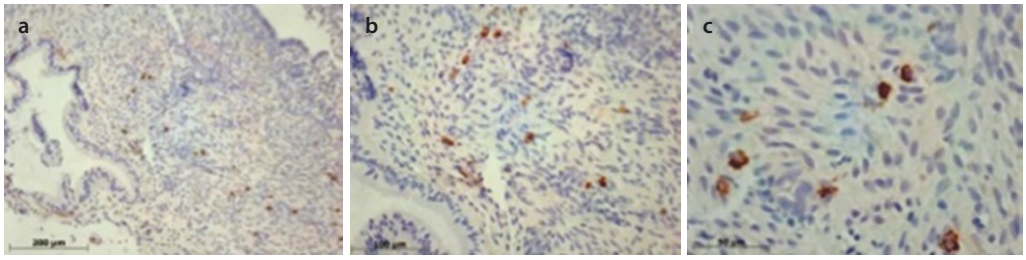


Fig. 10.10 Immunohistochemical evidence of chronic endometritis: immunohistochemical diaminobenzidine (DAB) staining for CD138-positive plasma cells in an endometrial biopsy. Magnifications: **a** × 100; **b** × 200; **c** × 400; DAB detection of CD138, counterstaining with hemalaun

ideal timing for hysteroscopy with endometrial biopsy has not been adequately evaluated to date.

In addition to immunohistochemistry, which requires an endometrial biopsy, hysteroscopy is another diagnostic procedure. Changes that should characterize a CE consist of diffuse hyperemia, hemorrhages, micropolyps (<1 mm) or mucous edema (Ettore Cicinelli et al. 2005).

Bouet et al. described a sensitivity of 40% and a specificity of 80% for hysteroscopy for the detection of CE (determined in 94 patients with implantation disorders or RPL) (Bouet et al. 2016). The authors point out that hysteroscopy, which is also significantly influenced by the clinical experience of the examiner, should only be used for orientation and cannot replace biopsy for the diagnosis of CE (Bouet et al. 2016).

In patients with ≥2 pregnancy losses before 20 weeks of gestation, the live birth rate in the subsequent pregnancy was 67.6% in the case of untreated CE and 87.1% without CE (McQueen et al. 2015). Recent studies show that there is a decrease in inflammatory response with 14–21 days of antibiotic therapy and increased chances of live birth in the subsequent pregnancy (El Hachem et al. 2017; Johnston-MacAnanny et al. 2010; McQueen et al. 2015).

Here, the broad-spectrum antibiotic doxycycline is preferred (El Hachem et al. 2017). Johnston-MacAnanny et al. reported that 70% of cases with histologically diagnosed CE were successfully treated after a 14-day course of 100 mg doxycycline twice daily (Johnston-MacAnanny et al. 2010). Kitaya et al. demonstrated successful therapy in 96% of cases with the same treatment regimen (Kitaya et al. 2012). Another study was able to demonstrate a histopathological cure rate of 94% after antibiotic therapy. Here, a combination therapy of ofloxacin (400 mg, two times daily for 14 days) and metronidazole (500 mg two times daily for 14 days), doxycycline alone or in combination with metronidazole, and a combination therapy of ciprofloxacin plus metronidazole were used (McQueen et al. 2014).

This study also showed that the live birth rate per pregnancy in RPL patients was 7% before treatment of CE and 56% after treatment (McQueen et al. 2014).

However, randomized, placebo-controlled prospective studies are still lacking.

Peripheral and Uterine Killer Cells

Killer cells are able to "kill" viruses and bacteria, but also tumor cells, and thus protect the organism. They can be detected both in the peripheral blood (peripheral NK cells, pNK cells) and in the endometrium (uterine NK cells, uNK cells). There are some functional differences between pNK and uNK cells, which are also reflected in the expression of various surface receptors (Fig. 10.11).

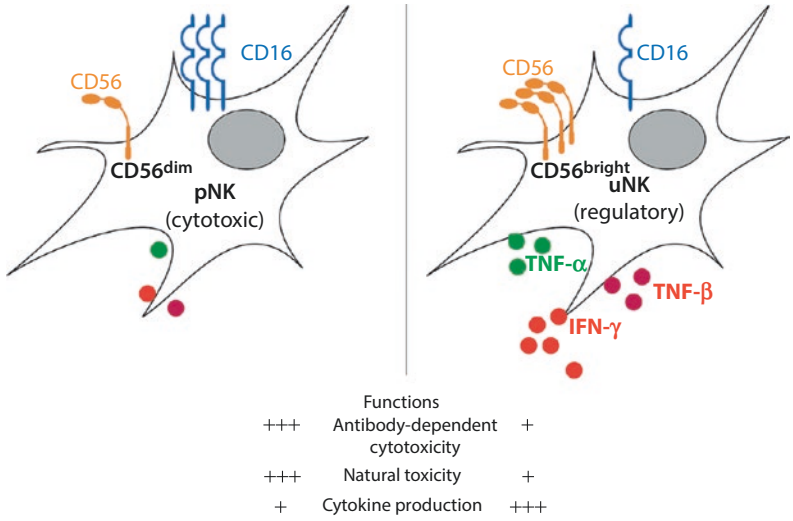


Fig. 10.11 Function, surface markers and cytokines produced by pNK and uNK cells. pNK cells show low cytokine secretion and thus lower regulatory components with high natural and antibody-dependent cytotoxicity. uNK cells take on mainly regulatory properties with a high cytokine secretion. *pNK* peripheral natural killer cells, *uNK* uterine natural killer cells, *TNF-α* tumor necrosis factor alpha, *TNF-β* tumor necrosis factor beta, *IFN-γ* interferon gamma

The pNK cells have predominantly cytotoxic properties, whereas uNK cells are predominantly cytokine-expressing and not very cytotoxic, and thus mainly deal with regulatory functions (Koopman et al. 2003; Lash et al. 2006). The recruitment of uNK cells during the increase in the luteal phase possibly occurs to a certain extent through a migration of pNK cells; in addition, there are also tissue-resident lymphocytes or stem cells, which can further differentiate into uNK cells. To date, no direct correlation between the concentration of pNK and uNK cells has been demonstrated in international studies.

NK cells are of central importance in (early) pregnancy because, among other things, they protect the fetus from pathogens, control trophoblast invasion and are significantly involved in the remodeling of spiral arteries. Therefore, increased levels of uNK and pNK cells are associated not only with pregnancy loss, but also with other obstetric complications such as maternal hypertension, preeclampsia, and fetal growth restriction (Koo et al. 2015; Lyall et al. 2013; Plaisier et al. 2009; Quenby et al. 2009). In summary, several international studies indicate that there is a subset of patients with RPL who have increased levels of pNK cells compared to healthy controls (Karami et al. 2012; King et al. 2010; Kuon et al. 2017a; Kwak et al. 1995; Seshadri and Sunkara 2014). According to studies on the presence of uNK cells in patients with RPL, the data is more inconsistent compared to pNK cells, mainly due to differences in the respective diagnostic methods used to detect uNK cells (immunohistochemistry vs. flow cytometry) (Clifford et al. 1999; Kamoi et al. 2015; Kuon et al. 2017b, c; Lachapelle et al. 1996; Michimata et al. 2002; Quenby et al. 1999; Shimada et al. 2004; Tuckerman et al. 2007). Overall, however, the current studies indicate that increased levels of uNK cells are present in patients with idiopathic RPL compared to healthy controls (Kuon et al. 2017c).

Despite the large amount of data on the occurrence of increased uNK and pNK cell

concentrations in RPL patients, there is currently no uniform international treatment concept. In the studies available to date, a variety of immunomodulatory therapies have been used. These include corticosteroids (pre-conceptional or in early pregnancy), lipid infusions (pre-conceptional or after a positive pregnancy test), immunoglobulins (in case of a positive pregnancy test) and other treatment strategies. The current guidelines therefore recommend immunomodulatory therapies in patients with RPL and elevated pNK and uNK cells only within clinical trials.

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Placental Insufficiency/ Placenta-Associated Diseases

Berthold Huppertz, Ulrich Pecks, and Holger Stepan

Contents

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11.1 Placental Disorders: Pathophysiology

Berthold Huppertz

11.1.1 Introduction

This chapter is dedicated to two of the major conditions that can negatively affect pregnancy: Preeclampsia and Fetal Growth Restriction (FGR). Both syndromes have in common that they are caused by placental dysregulation. However, both disorders also have in common that their pathophysiology remains unclear. Here, on the one hand, an attempt is made to name explanations for the pathophysiologies. On the other hand, hypotheses are presented that are based on current research findings.

11.1.2 Preeclampsia

Preeclampsia is the second most common cause of maternal morbidity and mortality worldwide, affecting approximately 2–7% of all pregnant women (Walker 2000; Roberts and Cooper 2001). About 18% of all deaths of pregnant women are due to preeclampsia (Davey and MacGillivray 1988; Roberts and Cooper 2001). It also contributes significantly to fetal and neonatal mortality and morbidity (Sibai et al. 1993; Levine et al. 1997).

Definition

According to the International Society for the Study of Hypertension in Pregnancy (ISSHP) (Davey and MacGillivray 1988; Lindheimer et al. 2009), preeclampsia is defined as the occurrence of a new hypertension of >140/90 mmHg (systolic/diastolic) after the 20th week of pregnancy in a previously unremarkable pregnant woman. In preeclampsia, this high blood pressure is coupled with an increased excretion of proteins in the urine, a proteinuria (>300 mg/dl). If the hypertension reaches values of >160/110 mmHg and/or the proteinuria values of >3 g/dl protein, this is referred to as severe preeclampsia.

Definition of Preeclampsia

A revised definition of pre-eclampsia came from the ISSHP in 2014 and includes the following criteria:

- **De novo hypertension after 20 weeks gestation** together with one or more of the following additional medical conditions:
  - Proteinuria
    - Urine protein/creatinine >30 mg/mmol (0.3 mg/mg) or
    - >300 mg/day or
    - at least 1 g/L [“2+”] in a urine dipstick measurement
  - Other maternal organ dysfunctions
    - Renal insufficiency (creatinine >90 µmol/l; 1.02 mg/dl)
    - Liver involvement (elevated transaminases—at least two times the upper limit of normal and/or pain in the upper right quadrant of the abdomen or epigastric abdominal pain)
    - Neurological complications (examples include eclampsia, altered mental status, blindness, stroke)
    - Hematologic complications (thrombocytopenia—platelet counts <150,000/dl, disseminated intravascular coagulopathy [DIC], hemolysis).
  - Uteroplacental dysfunction
  - Fetal growth restriction

Preeclampsia may progress and develop into eclampsia, which is characterized by the occurrence of tonic-clonic convulsions. These occur with or without loss of consciousness and occur postpartum (44%),

antepartum (38%) or intrapartum (18%). This makes eclampsia a life-threatening condition for the mother, which can have direct negative consequences for the child (Sibai 1990; Douglas and Redman 1994; Moodley and Kalane 2006).

Other conditions such as the HELLP syndrome ("hemolysis, elevated liver enzymes, low platelets") (Sibai 2004) and fetal growth restriction (FGR) may be associated with preeclampsia, further increasing the risk of maternal and fetal morbidity and mortality (Srinivas et al. 2009). As described above, FGR has now been included as a possible medical condition in the definition of preeclampsia.

Classification and Epidemiology

The further classification of preeclampsia is important because the different subtypes seem to have different pathophysiologies (Huppertz 2008). A general classification follows the gestational age at the time of delivery (von Dadelszen et al. 2003):

Classification of Preeclampsia According to the Date of Birth

- Early onset preeclampsia: delivery before the 34th week of pregnancy
- Preterm preeclampsia: delivery between the 34th and the 37th week of pregnancy
- Late onset preeclampsia: delivery after the 37th week of pregnancy until postpartum
- Preterm preeclampsia is often included in late onset preeclampsia, i.e.: delivery <34th week: early onset preeclampsia, delivery >34th week: late onset preeclampsia.

In doing so, the authors follow the approach of many clinicians who classify preeclampsia according to the time of delivery rather than the time of first onset of clinical symptoms. This latter timing is elusive, as it is unclear when a pregnant woman considers symptoms severe enough to seek medical attention.

Preterm preeclampsia at 34–36 weeks of gestation carries a 3.3-fold increased risk of mortality, while the risk of mortality from early onset preeclampsia before 28 weeks of gestation is as high as 12.5-fold (MacKay et al. 2001).

Based on data from the National Institute of Child Health and Human Development (NICHD) in the USA, 25–27% of women with preeclampsia require premature delivery. The earlier the birth, the more severe the complications for mother and child (Myatt and Miodovnik 1999). More than 50% of early onset preeclampsia cases require cesarean delivery, compared with only 30% of late onset preeclampsia cases, and compared with a cesarean section rate of 15–18% in the overall population (Douglas and Redman 1994).

Early onset preeclampsia is very rare (0.4–1.4% of all pregnant women), but has the highest rate of eclampsia and other severe maternal complications compared to late onset preeclampsia (Douglas and Redman 1994). This is equally true for neonatal complications, where the number of deaths worldwide due to preeclampsia is approximately 500,000. Here, the risk of growth restriction is significantly increased in early onset preeclampsia. This is associated with the risk of severe disabilities and cerebral palsy or even intrauterine fetal death.

Long-Term Effects

Women who have experienced preeclampsia are at increased risk of obesity, diabetes and/or cardiovascular dysfunction later in life (Smith et al. 2001). These women were found to have significantly increased rates of heart disease and death over a 15–19 year period following early onset preeclampsia in their

first pregnancy (Smith et al. 2001). In a comparison of deaths due to cardiovascular disease 30 years after preeclampsia, 85.9% of women after early onset preeclampsia, 98.3% of women after late onset preeclampsia, and 99.3% of women without preeclampsia were still alive (Mongraw-Chaffin et al. 2010). The risk of death from myocardial infarction is 5.1-fold higher after early onset preeclampsia and 1.6-fold higher after late onset preeclampsia (Irgens et al. 2001). The overall result is that women who have experienced early onset preeclampsia, in particular, have a significantly shortened life span of about 13 years after the first birth (Irgens et al. 2001).

So far, it is still open whether the link between preeclampsia and increased later risk of obesity, diabetes and cardiovascular disease and death is direct or indirect. There could be a direct link, and preeclampsia during pregnancy increases later risk. However, it could also be that the woman has an undiagnosed pre-existing condition which then leads to preeclampsia during the "stress test pregnancy." Thus, even without pregnancy and thus without preeclampsia, this woman would have an increased risk later in life.

The long-term effects not only affect the mother, but also the child is epigenetically reprogrammed by stress during pregnancy (Choudhury and Friedman 2012). This direct fetal programming is associated with an increased risk of children developing obesity and diabetes in adolescence (von Ehr and von Versen-Höynck 2016; Godfrey and Barker 2001).

Pathophysiology

Although massive efforts have been made in recent decades to elucidate the etiology of preeclampsia, this syndrome still remains what it has been for decades: a pathology of hypotheses.

A few points are clear and unambiguous: it takes the placenta, but not the fetus for preeclampsia. Molar pregnancies without a fetus can still develop preeclampsia. Once the placenta is born, the woman's symptoms disappear. Due to the many risks that can influence the development of preeclampsia, it is now assumed that it is a multifactorial event in which both the placenta and the predisposition of the woman play decisive roles. The interplay of these two factors determines the development of preeclampsia as well as its severity: early or late onset, mild or severe, etc.

Risk Factors for the Development of Preeclampsia

A variety of risk factors have now been described that increase a woman's risk of developing preeclampsia during pregnancy. These include in particular:

- previous preeclampsia, especially if it was severe or early onset preeclampsia (<34 weeks gestation),
- pre-existing underlying diseases such as chronic hypertension, kidney disease or diabetes mellitus,
- antiphospholipid antibody syndrome (APS),
- multiple pregnancies.

In addition, there are factors that are weaker but still clearly associated with the development of preeclampsia:

- First pregnancy (primipara; although preeclampsia may occur in subsequent pregnancies without preeclampsia being present in the first pregnancy),
- With >3 pregnancies the risk increases beyond that of the first pregnancy,
- First paternity (primipaternity or alternate paternity or a period of >5 years between two pregnancies with the same father),

- Short period of sexual intercourse (<6 months) before pregnancy,
- Obesity,
- Belonging to the African-American race,
- Advanced maternal age,
- Incidence of preeclampsia in the family.

Differences Between Early and Late Onset Preeclampsia

Early onset preeclampsia accounts for only about 10–15% of all preeclampsia cases, while preterm and late onset cases together account for >85%. At the same time, there are clear differences between early and late onset preeclampsia.

Late onset preeclampsia can generally be characterized as follows:

- The child displays normal height and weight.
- Blood flow in the uterine arteries is normal or only very slightly altered. Thus, there are no significant changes in Doppler ultrasound or pulsatility index.
- Blood flow in the umbilical arteries is unchanged.
- Women with increased placental mass/surface area (diabetes mellitus, multiple pregnancies, anemia, high altitude) are at increased risk for developing late onset preeclampsia.

Most **early onset preeclampsia** cases, on the other hand, have the following characteristics:

- The child is often too small and has a growth restriction.
- Blood flow in the uterine arteries is often altered. With this there are clear changes in Doppler ultrasound and increased pulsatility index.
- Increased peripheral resistance in the placental vessels could be a reason for the change in blood flow in the umbilical arteries. This may involve changes in blood flow in these vessels with flow still present (PEDF, preserved end-diastolic flow). Blood flow may stop at the end of diastole (AEDF, absent end-diastolic flow) or blood may even flow backwards through the umbilical arteries at the end of diastole (REDF, reversed end-diastolic flow).
- Trophoblast invasion is insufficient, especially with regard to endoarterial invasion into the spiral arteries.

However, it must be made clear that the characteristics "specific" to early onset preeclampsia are not specific to this syndrome! All the characteristics listed above for early onset preeclampsia also apply to early fetal growth restriction (FGR), which is not associated with maternal symptoms. The **typical characteristics of early FGR** are:

- Of course, a child with a growth restriction,
- Inadequate trophoblast invasion, especially into the spiral arteries,
- Change in blood flow through the uterine arteries (increased pulsatility index),
- Changes in blood flow through the umbilical arteries (PEDF via AEDF to REDF).

Since the two early syndromes, early onset preeclampsia and early onset FGR often occur in parallel, the question must be asked whether they are directly associated with each other or whether, under certain circumstances, they occur either alone or in parallel. There is also an attempt in the literature to explain that one syndrome (early onset preeclampsia) is responsible for the second syndrome (early FGR). Of course, this can only apply to cases where both occur in parallel. Unfortunately, clarification is not yet in sight.

Presentation and Falsification of the Outdated Hypothesis on the Etiology of Preeclampsia

Interestingly, a hypothesis on the etiology of preeclampsia, which uses the deficient invasion of the extravillous trophoblast as a

basis, has been described in the scientific literature for decades. It has long since been disproved by a large number of studies. The continued citation as one of the most important hypotheses demonstrates the importance of a critical examination of hypotheses. At the same time, it also shows that the falsification and rejection of a hypothesis as the most important tool of science does not necessarily lead to its abandonment.

However, since the said hypothesis is still being circulated today, here is a brief summary of the chronological sequence of events described in this hypothesis:

- First trimester: a previously undescribed deleterious effect on the extravillous trophoblast.
- First trimester: Deficient invasion of the extravillous trophoblast with a markedly reduced invasion of the spiral arteries in the first and second trimester.
- Second trimester: reduced flow of maternal blood into the intervillous space of the placenta.
- Second and third trimesters: placental hypoxia or events of hypoxia followed by reoxygenation of the placenta.
- Second and third trimester: hypoxic damage to the villous trophoblast.
- (Second and) third trimester: release of placental factors of the syncytiotrophoblast (such as sFlt-1 and PlGF) into the maternal blood.
- (Second and) third trimester: maternal inflammatory reaction due to these placental factors and development of the mother's clinical symptoms.

This was a conclusive hypothesis over 10 years ago that could explain many aspects of preeclampsia. It has since been refuted in many places. It is therefore all the more surprising that it is still cited and supported. In the following, two events crucial to this hypothesis will be considered in more detail.

Deficient Trophoblast Invasion and Preeclampsia

In the scientific literature in connection with the pathophysiology of preeclampsia in general, one almost always finds the change in the extravillous trophoblast that is only known for early onset preeclampsia: deficient trophoblast invasion. Considering that early onset preeclampsia accounts for only 10–15% of all preeclampsia cases, it must be asked how this came about and how the etiology of the other 85–90% of preeclampsia cases is to be explained.

Since early onset preeclampsia has proportionally the highest number of cases with severe complications and is also clinically extremely relevant due to the early gestational age at birth, such early onset preeclampsia cases have received and continue to receive significantly more attention and thus more research than the late cases—even though the latter occur five to six times more frequently. Early onset preeclampsia cases are often associated with FGR, which is why studies of early onset preeclampsia have led to the erroneous conclusion that these changes apply to all preeclampsia cases. However, since pure FGR cases also show the symptoms described above without the mother becoming symptomatic, it must be critically questioned what the true pathophysiology of early onset preeclampsia is.

This criticism is supported by studies on the prediction of preeclampsia using uterine Doppler ultrasound as a surrogate for deficient trophoblast invasion. Measurements of an elevated pulsatility index in the uterine arteries at 11 + 0 to 13 + 6 weeks gestation predicted preeclampsia (all cases) in only 40% with a 10% false positive rate (Nicolaides et al. 2006). Another study also showed a clear picture: here, too, blood flow in the uterine arteries was measured with Doppler ultrasound at the 11th–14th week of pregnancy. Based on the corresponding data, the authors were able to predict 21%

of all preeclampsia cases and only 33% of early onset preeclampsia cases. This could be expected against the background described above. At the same time, however, the authors were able to predict 100% of all early onset FGR cases (Pilalis et al. 2007).

Such and further studies increase the doubt that the deficient invasion of the extravillous trophoblast is causally involved in the etiology of preeclampsia. The dysregulation of the extravillous trophoblast seems to result mainly in fetal growth restriction, which occurs even in the absence of preeclampsia. Thus, the above-mentioned characteristics of early onset preeclampsia would be due to early onset FGR. And thus, up to this point, there would be no specific characteristics of early onset preeclampsia that could distinguish it from late onset preeclampsia—except for its co-occurrence with early onset FGR, which usually necessitates early delivery.

Deficient Placental Perfusion, Placental Hypoxia and Preeclampsia

As already described in ▶ Chap. 1, during the first trimester there is only a maternal plasma flow passing through the placenta. Thus, only oxygen physically dissolved in the fluid with a partial pressure of <20 mmHg is found in the placenta and also in the embryo (Jauniaux et al. 2000). This low partial pressure of oxygen is normal for this period of pregnancy and for this organ (placenta) and must not be confused with a deficient supply or even hypoxia.

Only with the onset of maternal blood flow into the placenta at the beginning of the second trimester does the intraplacental partial pressure of oxygen rise to about 60 mmHg (Rodesch et al. 1992; Jauniaux et al. 2000). An increase in placental oxygen partial pressure that occurs too early, can lead to loss of placental mass and even spontaneous pregnancy loss (Jauniaux et al. 2000, 2003).

At the same time, it must be taken into account that the tissues of the maternal uterus are oxygenated normally throughout pregnancy, i.e. at about 90 mmHg in the arteries and at about 40 mmHg in the veins (Jauniaux et al. 2000). In these uterine tissues, the partial pressure of oxygen does not change throughout pregnancy, so that invasion of the extravillous trophoblast always occurs into a well-oxygenated uterine tissue (Huppertz et al. 2009). Thus, deficient trophoblast invasion cannot lead to changes in the partial pressure of oxygen in the placental bed, i.e. in the uterine wall. However, it can influence the flow of maternal blood into the placenta.

The steep increase in intraplacental partial pressure of oxygen at the beginning of the second trimester is followed by a slight decrease until the end of pregnancy. The values measured over the second half of pregnancy until delivery range between 30 and 50 mmHg (Schaaps et al. 2005; Sibley et al. 2002; Kakogawa et al. 2010).

These oxygen partial pressures are ensured by the dilatation and further remodeling of the uterine spiral arteries through the invasion of the extravillous trophoblast (Kaufmann et al. 2003; Pijnenborg et al. 2006). Importantly, this dilatation mainly affects the end of the spiral arteries at the transition to the placenta. This leads to a funnel-shaped opening of these arteries towards the intervillous space of the placenta.

At the same time, because of the growth of the fetus and placenta, the amount of blood flowing into the placenta is increased. From the 20th–38th week of pregnancy alone, the uterine blood flow increases from about 450 ml/min to almost 1000 ml/min (Konje et al. 2001).

What Is the Effect of the Funnel-Shaped Dilatation of the Uterine Arteries?

The blood flow velocity through the uterine arteries is relatively constant in the second

half of pregnancy, with values between 33 and 50 cm/s (Bahlmann et al. 2012). Burton et al. (2009)—based on appropriate assumptions such as length and diameter of the vessels, total blood volume and others—have calculated the velocity at which maternal blood eventually flows into the placenta. The funnel-shaped dilatation at the end of the vessels reduces the velocity of maternal blood flow into the placenta to about 10 cm/s (Burton et al. 2009). This low flow velocity does not destroy the very fragile villous tree structures, allows blood to be distributed through the narrow passages between villi, and does not cause anchoring villi to break away from the basal plate.

What Is the Effect of Deficient Trophoblast Invasion?

Blood flow from opened but not dilated spiral arteries into the placenta has a velocity of about 1–2 m/s according to calculations by Burton et al. (2009), which is 10–20 times faster than in normal pregnancy. This has a dramatically negative effect on the villous trees of the placenta that is not associated with a change in oxygen partial pressure. Rather, the increased inflow velocity leads to damage to the villous surface (increased deposition of fibrin type fibrinoid), rupture of anchoring villi from the basal plate (further reducing the number of extravillous trophoblasts), and changes in the overall structure of the villous trees. Thus, the supply of the fetus is significantly impaired and a deficient supply of the fetus resulting in FGR may well be the consequence.

Does This Really Lead to Deficient Placental Perfusion and Placental Hypoxia?

Interestingly, hundreds of publications can be found that use both deficient perfusion and placental hypoxia as the basis for their data. At the same time, there is still no measurement that can prove either of the two processes. If we look at placental oxygenation in preeclampsia and FGR, we only find a few published values:

- Measurements of the partial pressure of oxygen (pO_2) in blood samples of the uterine veins during a cesarean section revealed a mean value in early onset and preterm FGR pregnancies of 63 mmHg (birth between the 24th and 36th week of gestation). Calculated back to the pO_2 in the placenta, this results in a value of 42 mmHg, which is 1.3 times higher than the value of the healthy cohort in this study (Sibley et al. 2002).
- Measurements of oxygenated hemoglobin concentration have also been used as a surrogate for placental tissue pO_2. If the tissue oxygenation index of the placenta was 71% in healthy pregnant women, this value increased to 78% in cases with FGR. In cases with early onset preeclampsia and FGR, the values even increased to 80%. Transferred to intraplacental pO_2–values, this results in a partial pressure of oxygen for normal pregnancies of about 30 mmHg, whereas in cases with FGR values of 49 mmHg and in cases of early onset preeclampsia with FGR values of 54 mmHg were present (Kakogawa et al. 2010; Kawamura et al. 2007).

In summary, measured values of the partial pressure of oxygen in the placenta, which prove placental hypoxia in preeclampsia, are not yet available. On the contrary, there is increased evidence of elevated pO_2 values in the placenta in cases with deficient trophoblast invasion. This was postulated for cases with FGR (with and without preeclampsia) 20 years ago (Kingdom and Kaufmann 1997) and has now been demonstrated with measurements in pregnant women (Sibley et al. 2002; Kawamura et al. 2007; Kakogawa et al. 2010). Moreover, no evidence of deficient trophoblast invasion is found in >80% of all preeclampsia cases. Here, the hypothesis described above does not apply at all.

Possible Explanation of the Etiology of Preeclampsia

As described above, the placenta is inevitable for the development of preeclampsia. At the same time, the placenta cannot be considered alone here, but must always be analyzed in the context of its environment. Only the interaction between placenta, release of placental factors into the maternal circulation, and maternal response to these factors will determine whether or not a pregnant woman will develop preeclampsia. Therefore, the effect of each side must be investigated in order for symptoms to become clinically relevant (Huppertz 2008).

Given the close interactions between maternal and fetoplacental factors during pregnancy, at least three scenarios can be considered. These different scenarios can explain the different types and manifestations of preeclampsia, the different times of onset and the different effects on mother and child in later life (◘ Fig. 11.1):

▪ Scenario 1a: A Healthy Mother with a Malfunction of the Placenta in the Villous Trophoblast

During normal pregnancy, the syncytiotrophoblast releases factors by secreting substances in a controlled manner or by releasing them as syncytial knots via apoptotic processes (Huppertz et al. 2006; Huppertz 2008, 2010). In this scenario, dysfunction of the syncytiotrophoblast results in subcellular material entering the maternal circulation through necrosis and aponecrosis (Huppertz et al. 2006; Huppertz 2008, 2010). This release of subcellular particles such as micro- and nanoparticles (Johansen et al. 1999; Redman and Sargent 2000) systemically activates and permanently damages the maternal endothelium (Goswami et al. 2006). Dysfunction of the placenta and specifically the syncytiotrophoblast leads to the ongoing release of factors that can activate and/or damage the maternal vasculature and thus induce preeclampsia.

The extent of this damage can be directly correlated with the surface area of the placenta: The greater the placental surface area (e.g., in large placentas [diabetes], in multiple pregnancies, or pregnancies at high altitude), the greater the amount of non-apoptotic factors released. In addition, the maternal defense system is overloaded above a certain amount of factors, and thus damage to the maternal endothelium is possible even faster.

If the maternal defense system can withstand the amount of placental factors over a longer period of time, the clinical symptoms of preeclampsia will develop late (late onset preeclampsia). Since this implies that the maternal endothelium also suffers damage late in pregnancy, predictive markers that represent damage to the endothelium (such as the angiogenic factors sFlt-1 and PlGF) will not show changes before the onset of clinical symptoms. Moreover, angiogenic factors are more related to FGR (Nicolaides et al. 2006; Pilalis et al. 2007). At the same time, placenta-specific predictive markers such as PP13 show changes in their release pattern from the syncytiotrophoblast already in the first trimester (Huppertz et al. 2008).

In this scenario, a normal-weight child usually develops without growth restriction. However, damage to the syncytiotrophoblast can also negatively affect the absorption of nutrients, so that it cannot be ruled out that at least late effects are possible for the child in adulthood. After birth, the mother should recover completely and not suffer any long-term consequences.

▪ Scenario 1b: A Healthy Mother with a Malfunction of the Placenta in the Extravillous Trophoblast

If there is a defect of the extravillous trophoblast in the placenta, the release of factors from the syncytiotrophoblast is not altered. In this case, however, there is

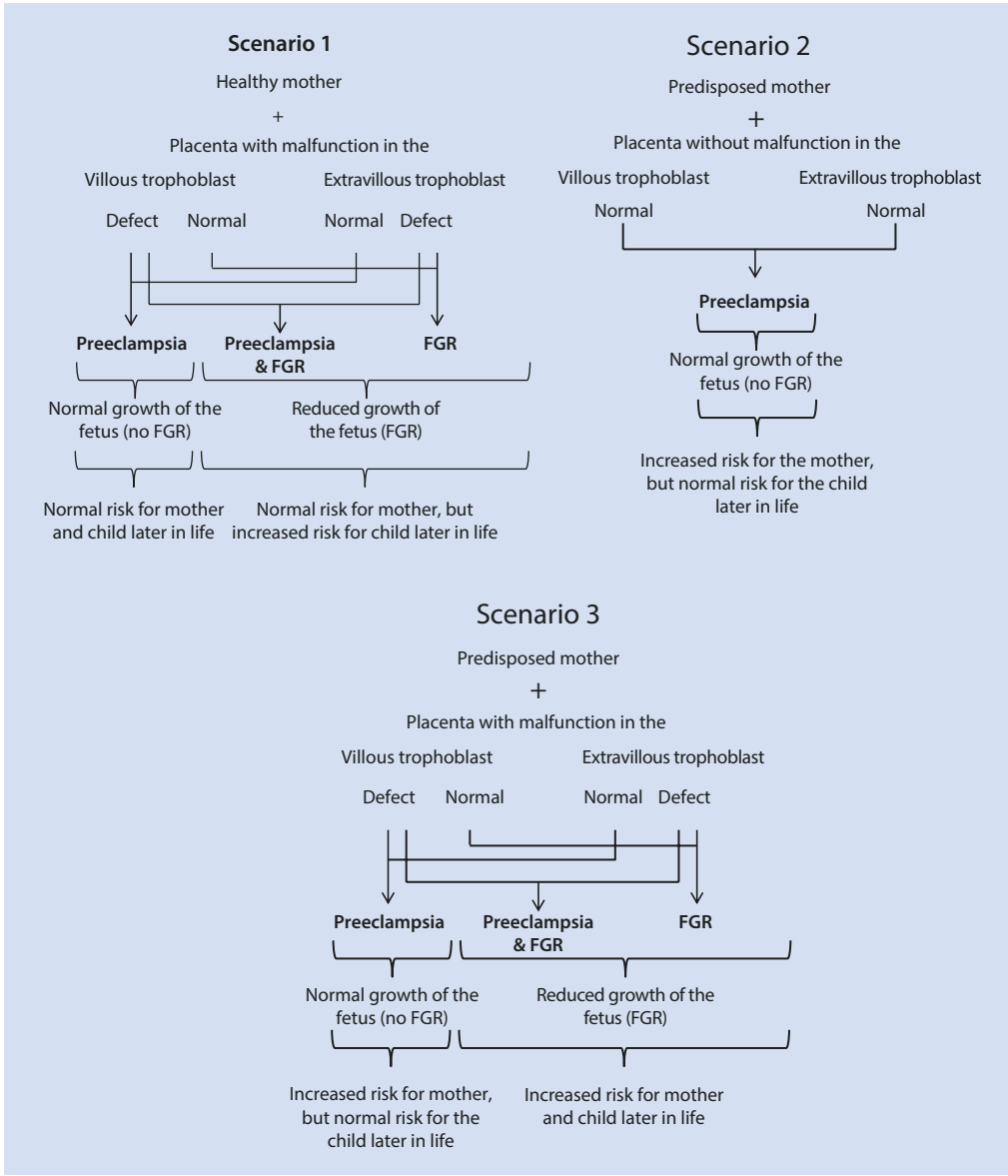


Fig. 11.1 Schematic representation of the scenarios that can lead to preeclampsia and/or fetal growth restriction (FGR). Scenario 1 describes the effects on mother and child in the case of a healthy mother and a malfunction in the placenta. Scenario 2 shows the effects on mother and child in a pre-diseased mother and a healthy placenta, while Scenario 3 shows the effects on mother and child in a pre-diseased mother and a malfunctioning placenta

deficient invasion of the extravillous trophoblast with subsequent altered delivery of maternal blood to the placenta. One effect of the increased flow velocity of the maternal blood is an increase in peripheral resistance in the small vessels of the placental villi. This increased resistance can lead to changes in fetal blood flow to the placenta, resulting

in poorer delivery of both nutrients and oxygen to the fetus.

Evidence of arrest or reflux of blood flow in the umbilical arteries at the end of diastole (AEDF or REDF) reveals corresponding serious consequences for the fetus. In these situations, a hypoxic fetus may be associated with a hyperoxic placenta: The same amount of blood (and therefore oxygen) flows into the placenta from the maternal side. Due to the increased flow velocity in the placenta and the damaged villous surface, the placenta absorbs less oxygen and passes less oxygen on to the fetus. Thus, the fetus develops a hypoxia while there is a higher partial pressure of oxygen in the placenta compared to the normal placenta. Hyperoxia occurs in the placenta in these cases of FGR.

The following scenario has not yet been clarified and can only be depicted hypothetically:

The alteration of the maternal blood flow in the placenta and the damage to the villous structure may subsequently also lead to damage of the villous surface and thus to a release of subcellular material from the syncytiotrophoblast. Thus, in this scenario, the deficient trophoblast invasion could cause FGR of the fetus and subsequently also induce preeclampsia.

In this scenario, a child develops with growth restriction and it cannot be ruled out that late effects in adulthood are possible. If the mother does not develop preeclampsia, she should not suffer any long-term consequences.

Scenario 2: A Predisposed Mother with a Normal Placenta

In this scenario, the placenta releases the normal portfolio of fragments, factors and molecules into the maternal circulation. However, pre-existing damage in the mother implies that the "pregnancy stress test" is not without complications for the mother. The pre-existing damage may be to the woman's defense system, to the endothelium itself, or to signaling cascades that regulate renal function and blood pressure (e.g., chronic hypertension, antiphospholipid syndrome, etc.). Ultimately, this damage causes the overall system of a pregnant woman to respond inadequately to placental factors, resulting in the development of clinical symptoms of preeclampsia. This is true even if the quality and quantity of placental factors are within the normal range.

Also in this scenario, the extent of preeclampsia can be correlated with the surface area of the placenta. The greater the placental surface area, the greater the amount of apoptotic factors released. Depending on the damage to the maternal system, the system may become overloaded early (early onset preeclampsia) or only towards the end of pregnancy (late onset preeclampsia), leading to the expression of clinical symptoms.

For predictive markers released from the maternal side, changes could occur early and thus indicate an increased risk for the woman. At the same time, no difference will be seen in placenta-specific markers, as the placenta does not show any changes.

In this scenario, most often a normal weight child develops without growth restriction and without expected late effects in adulthood. This looks different in the mother. Since the woman already suffers from a subliminal and subclinical disorder, this can lead to a higher risk of cardiovascular disease later in life.

Scenario 3: A Predisposed Mother with Placental Dysfunction

This scenario contains the most severe cases, as the combination of defects on both sides can lead to severe symptoms in the child, but especially in the mother.

On the placental side, different subscenarios can be described.

- If the villous trophoblast alone is affected, damage to the villous surface

will occur, resulting in the release of subcellular particles from the syncytiotrophoblast into the maternal blood. These necrotic particles will then encounter an already pre-damaged vascular system of the mother. Thus, the clinical picture of preeclampsia will develop early in these women without the need for growth restriction of the fetus. Such cases should be predictable with placenta-specific markers such as PP13 as well as with vascular-associated markers such as sFlt-1 and PlGF.

- If not only the villous but also the extravillous trophoblast is affected in the placenta, deficient invasion also occurs (Huppertz 2011). In these cases, the syncytiotrophoblast releases necrotic factors and the deficient invasion alters blood flow through the placenta (Burton et al. 2009). This can lead to growth restriction of the child, but in addition can also increase the extent of syncytial changes. In this worst case scenario, early severe preeclampsia of the mother with associated early onset FGR of the child may occur. These cases can be detected with all predictive markers, both placenta-specific (Chafetz et al. 2007) and angiogenic markers (Schaarschmidt et al. 2013).

In this third scenario, mothers are at significantly increased risk of cardiovascular disease and other conditions later in life. In cases with FGR, the children will also suffer long-term damage from fetal programming (Longtine and Nelson 2011; Hogg et al. 2013).

All these different scenarios make it clear that the origin and etiology of preeclampsia will not be clarified for a long time yet. New approaches and explanatory models must be found through appropriate research actions in order to finally come closer to clarifying the origin of preeclampsia.

11.2 FGR: Diagnostics and Management

Ulrich Pecks

Fetal growth restriction (FGR) occurs when a fetus is unable to reach its genetically predetermined growth potential. The reduced growth rate results in a low birth weight usually below the tenth percentile. Antenatal sonography and risk history help in correct diagnosis in differentiation from constitutionally small-for-gestational-age (SGA) fetuses and in further assessment of outcome. The challenge for the obstetrician is to determine the optimal time of delivery in order to avoid fetal death while minimizing morbidity and mortality associated with preterm birth.

11.2.1 Terminology and Definition

The terminology used in the literature for children born small or light is often not very clear-cut. "Low birth weight" (LBW), "small for gestational age" (SGA) and "intrauterine or fetal growth restriction" (FGR/FGR) are often used synonymously.

> A birth weight <2500 g is referred to as Low Birth Weight (LBW), and a birth weight <1500 g is referred to as Very Low Birth Weight (VLBW), regardless of the week of gestation or underlying cause.

The term LBW was coined around 1930 by the Finnish pediatrician Yllpö and was used as an indicator of a short gestational period. It took into account the observation that these children required increased medical attention and care. Following epidemiological studies describing that the morbidity and mortality of a newborn with LBW depended significantly on the maturity of the child

(Van den Berg and Yerushalmy 1966), a distinction was made by the WHO in 1961 between preterm and term LBW (corresponding to 37th weeks of gestation). It quickly became apparent that gestational age and fetal weight affected neonatal outcome differently. In particular, preterm infants with <2500 g cannot be considered as a homogeneous group because of the different neonatal courses. From this, the need for weight percentile curves was born. Statistical models consider the population-based normal distribution of birth weight at a specific gestational age. For Germany, for example, the population-based percentile curves according to Voigt are available (Voigt et al. 1996). A deviation from the expected birth weight or its anticipated variance suggests abnormal fetal growth. It is controversially discussed at which percentile a deviation from the norm may be assumed and at which point this also implies clinical relevance (Lee et al. 2003). The tenth percentile is commonly used as a cut-off for the description of an SGA child. This describes a newborn who belongs to the group of children who were lighter than 90% of children of the same gestational age at birth at a defined gestational week. However, the fifth and third percentiles are also under discussion (Unterscheider et al. 2013a).

Infants with a birth weight below the tenth percentile for a given gestational age are defined as "Small for Gestational Age" (SGA). The term is descriptive and does not suggest pathology.

What remains unconsidered in population-based percentile curves are individual influences on birth weight. Genetic factors contribute to 30–50% of the variance in birth weight (Svensson et al. 2006; Lunde et al. 2007). Clinical characteristics with demonstrable influence on child weight include maternal height and weight, parity, and ethnicity, in addition to child sex. Optimized growth curves take these factors into account and are referred to as personalized ("customized") percentile curves.

Figure 11.2 shows an example of the fetal weight percentile curves of two patients of different origin, body measurements and parity. In both of them an ultrasound was performed in gestational week 36 + 4 with the result of a female fetus with an estimated weight of 2500 g. While in Ms. Short (bottom picture) adequate fetal growth is recorded in the 40th percentile, in Ms. Tall (top picture) there is an SGA fetus in the fifth percentile. A classification of the birth weight according to personalized percentiles increases the statement regarding the degree of risk of an SGA newborn (Gardosi et al. 2009).

Tip

Personalized percentile curves ("customized percentiles") can also be calculated for German-speaking countries on the online platform ► www.gestation.net.

While terms described above are purely descriptive of a child's postpartum status, the term FGR includes functional aspects related to antenatal growth dynamics.

Fetal growth restriction (FGR) occurs when a fetus is unable to reach its genetically predetermined growth potential during pregnancy.

Strictly speaking, the term retardation (lat. "retardare," to delay), which is often found in the literature, is not correct here, since in the narrower sense there is no delay but a restriction (lat. "restringere," to restrict) of fetal growth.

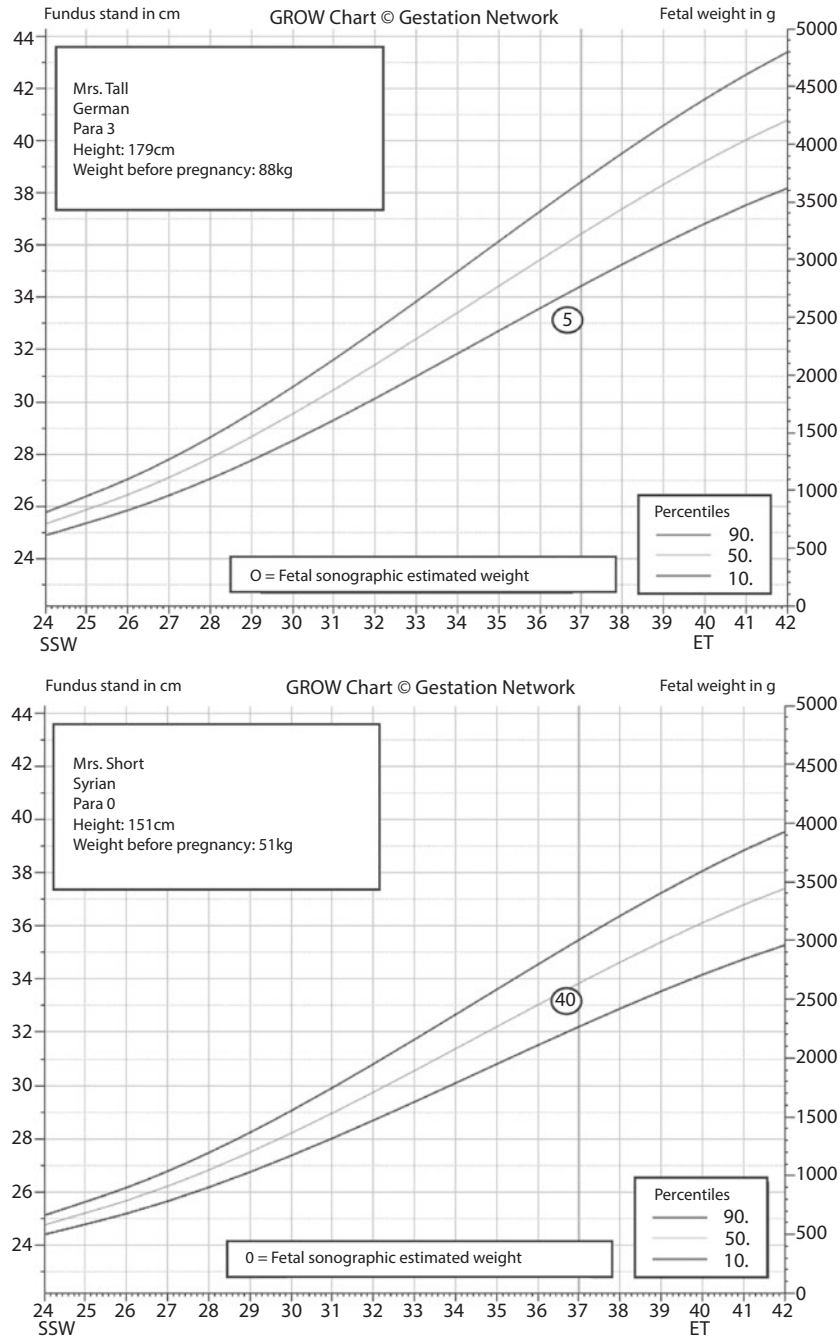


Fig. 11.2 Fetal weight percentile curves of two patients of different origin, body measurements and parity at 36 + 4 weeks gestation with female fetuses and an estimated fetal weight of 2500 g. Ms. Short *(bottom)* shows adequate fetal growth in the 40th percentile, while Ms. Tall *(top)* has an SGA fetus in the fifth percentile. (Personalized percentile curves courtesy of Prof. Jason Gardosi, Perinatal Institute Birmingham, UK; © Gestation Network—► www.gestation.net)

11.2.2 Epidemiology

In Germany, approximately 53,000 children (7.22%) were born with LBW in 2015. By definition, 10% of all children are born as SGA (<10th percentile). The population-based weight percentile curves for Germany were reviewed the last time in 2014 and have remained almost unchanged in validity over the past 20 years (Voigt et al. 2014). In numbers, there are about 70,000 SGA children per year. According to estimates, about 50–70% of all SGA newborns are constitutional, i.e. without any underlying pathology. The incidence of FGR is 3–8% of all pregnancies, depending on the definition and region used. FGR is a heterogeneous clinical picture that can be classified on the basis of clinical and pathophysiological features. The type, timing and duration of the pathological insult determine the severity and manifestation.

11.2.3 Cause and Risk Factors

In principle, fetal, maternal and placental causes of FGR can be distinguished, while the boundaries are often fluid or overlapping. Nevertheless, these different primary pathomechanisms usually (not always) lead to the same secondary phenomenon: suboptimal uteroplacental perfusion and fetal nutrition. ◘ Figure 11.3 illustrates the factors associated with reduced fetal growth.

Chromosomal disorders or infectious diseases each contribute to about 10% of all cases of the clinical picture of FGR (Khoury et al. 1988; Longo et al. 2014). Although rare, they are relevant in that the outcome is primarily determined by the underlying disease. This necessitates an individual approach to education, observation and treatment.

Tip

The online inheritance-in-man (OMIM) database contains numerous genetic syndromes that may be associated with FGR.

11.2.4 Fetal Compensation

The timing of the occurrence of an insult or the failure of compensatory mechanisms is important because the fetus goes through different developmental phases in the course of pregnancy. Hyperplastic growth dominates during the first 16 weeks. Growth inhibition during this period is more likely to result in symmetrical FGR, type 1 (approximately 30%). Above all, chromosomal aberrations, fetal malformations, infections (e.g. cytomegalovirus, CMV) and substance abuse (drugs, alcohol) are causatively attributed to type 1 FGR.

In contrast, a growth phase increasingly characterized by hypertrophy follows after the 24th week of gestation. A pathological insult is more noticeable at this time by an asymmetrical appearance (type 2 FGR, about 70%) (Ott 1988). Nutritive insufficiency leads to a redistribution of the fetal circulation. The proportion of blood supplied directly to the heart from the umbilical vein via the ductus venosus (normally 18–30%) increases (Haugen et al. 2004; Kiserud et al. 2006). Due to the acceleration of blood flow in the ductus venosus, more nutrient-rich blood from the umbilical vein passes through the foramen ovale into the left atrium of the heart. From there, it supplies the heart via the coronary vessels and the brain via the preductal aorta. This is done at the expense of less vital organs, such as the kidneys, lungs, or liver. Bypassing the liver leads to a decrease

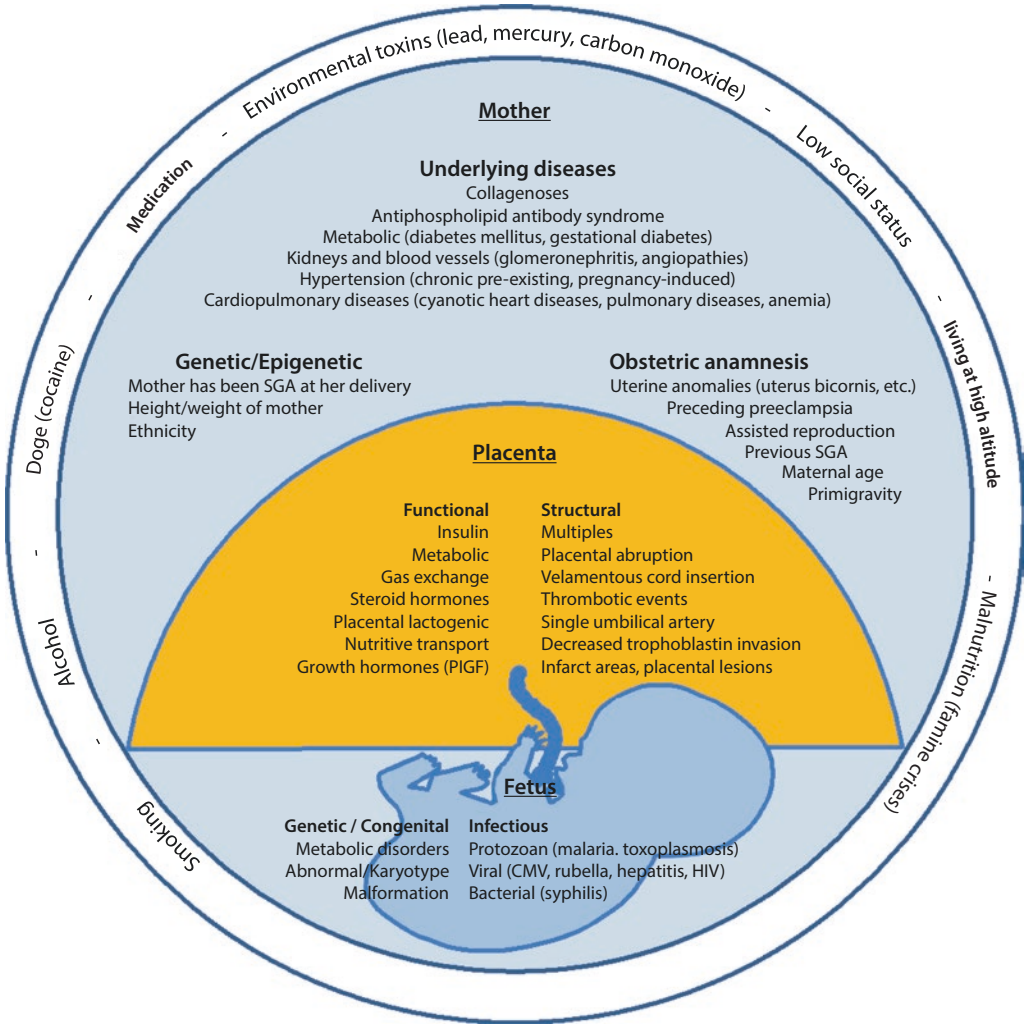


Fig. 11.3 Factors affecting intrauterine growth may be attributed to the mother, the placenta, or the fetus. Environmental and lifestyle factors are considered separately (outer ring). (Modified according to Gembruch et al. 2013)

in fetal glycogen reserves and liver volume. Consecutively, abdominal cirumference falls to a lower percentile with mostly maintained growth of the head ("brain-sparing effect"). If the placental insufficiency persists, the entire fetal biometry is affected in the further course, now with a symmetrical decrease of the total body growth. The redistribution of fetal blood flow can be observed by Doppler ultrasound. First changes with resistance increase of the fetal vessel wall can be detected in the arterial system, the umbilical arteries or the descending aorta. With increasing hypoxia, a decrease of the pulsatility index in the cerebral circulation follows (Arbeille et al. 1995). Increasing decompensation eventually leads to pathological venous flow patterns (Hofstaetter et al. 2002). In the ductus venosus, the pulsatility index for veins

(PIV) increases to the point of flow reversal during the so-called a-wave, in which atrioventricular inflow is reflected (Kiserud et al. 2006). Once the fetus' adaptive mechanisms to its nutritive insufficiency are exhausted and the fetus is no longer able to maintain this central redistribution, signs of the so-called "late cardiovascular response" occur, with a decrease in cardiac ejection, multiphasic venous patterns, and possibly a normalization of the middle cerebral artery (Ferrazzi et al. 2002). With increasing hypoxia and deterioration of the acid-base balance, the fetus responds with behavioral mechanisms. There is a decrease in overall activity and respiratory movements to complete inactivity with abolished flexion of the extremities (Manning 2002; Frøen et al. 2008). The fetal cardiac tone curve shows nonreactivity, which can be seen on cardiotocography (CTG) and Oxford CTG (Arabin et al. 1988; Visser et al. 2016).

On the basis of different clinical courses and compensation patterns depending on the gestational age, a distinction is increasingly being made between an early ("early onset") and a late ("late onset") form of FGR. Early forms of FGR are more often associated with an increased resistance index (RI) of the umbilical arteries up to end-diastolic absent or reversed flow (ARED flow) (Baschat 2011). Using a cohort of 656 FGR singletons, Savcheva et al. found that the 32nd week of gestation best distinguished between an early and late form of FGR using Doppler sonographic parameters of the umbilical arteries (Savchev et al. 2014). This is consistent with observations from our own cohort of 95 FGR singletons (Fig. 11.4).

A pathological Doppler of the umbilical arteries is the best discriminating prognostic factor with regard to fetal outcome.

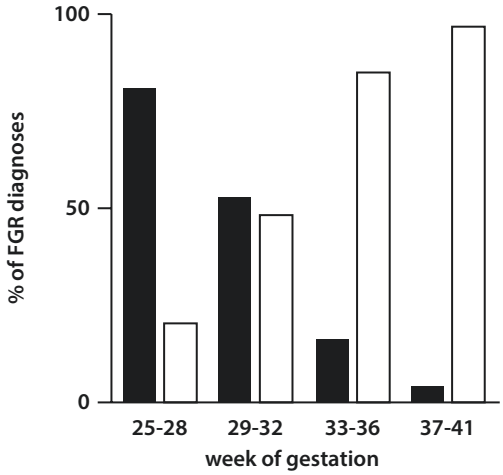


Fig. 11.4 Classification of FGR cases with and without ARED flow of the umbilical arteries. Collective of 95 cases who participated in a study at the University Women's Hospital of RWTH Aachen in 2006–2014 (Pecks et al. 2012, 2016). Shown is the percentage of patients with ARED *(black bars)* and without ARED *(white bars)* in their gestational age group

11.2.5 Outcome of the Child

It is undisputed that FGR can be held responsible for the majority of intrauterine fetal deaths (IUFD) (Hübner et al. 2015). According to an English study of 2625 stillbirths, FGR accounted for 42% of IUFD. In comparison, congenital anomalies accounted for the second largest proportion of all IUFD at 17% (Gardosi et al. 2014).

Based on a survey of infants born in the United States in 2005, the risk of IUFD in SGA infants increases as the percentile group decreases. Using population-based growth curves, fetuses <3rd percentile have an approximately tenfold increased risk of IUFD throughout gestation. After the 39th week of gestation, the relative risk increases substantially; thus, from the 41st week of gestation, it is 58 times (95% CI:

42.84–73.13) compared to that of the >10th percentile reference group (Pilliod et al. 2012). At a fetal weight <10th percentile, the risk of IUFD is still doubled (Getahun et al. 2007).

A prospective English survey identified a relative risk of IUFD in children classified as SGA <10th percentile according to personalized percentiles of 6.8-fold (95% CI: 5.6–8.4) compared with children >10th percentile (total population: 4.2 stillbirths per 1000 births). The proportional risk was fivefold higher when SGA was unknown antenatally (Gardosi et al. 2013). The implementation of programs to improve antenatal detection of FGR using personalized growth curves led to a lower rate of IUFD in regions with participating centers in England (Gardosi et al. 2014).

The outcome of the neonatal period is largely determined by three independent factors:

- the fetal age at birth,
- the fetal weight and
- the underlying pathology.

Up to 32 to 34 weeks of gestation, the fetal stage of development (prematurity) significantly determines the neonatal course (Baschat 2011). In addition, a low weight percentile has also been confirmed as an independent risk factor for neonatal morbidity. This particularly relates to intracranial hemorrhage and necrotizing enterocolitis (McIntire et al. 1999; Dashe et al. 2000; Aucott et al. 2004). An association with respiratory, hepatic and metabolic disorders is under debate (Hay 2006; Pike et al. 2012; Cianfarani et al. 2012; Pecks et al. 2012, 2014, 2016). Additive to low estimated weight, other antenatal parameters also have predictive value for the newborn. In a retrospective cohort study from the USA using 1364 SGA infants <10th percentile, a higher adverse composite outcome was observed in asymmetric SGA infants than in symmetric SGA infants (14% vs. 5%). This may be due to the fact that the asymmetrical SGA children were subject to FGR, whereas the symmetrical children were predominantly constitutional SGA (Dashe et al. 2000). In the PORTO study, a large Irish multicenter study (Prospective Observational Trial to Optimize Pediatric Health in Intrauterine Growth Restriction) that included over 1100 women with fetal estimated weight <10th percentile, infants with estimated weight <3rd percentile had the greatest risk of adverse outcome compared to those between the 3rd and 10th percentiles (6.2% vs. 2%). The highest risk factor for adverse perinatal outcome and more frequent transfer of the infant to a neonatal intensive care unit is a pathological fetal Doppler ultrasound finding (Vergani et al. 2005; Unterscheider et al. 2013a; Savchev et al. 2014; Khalil et al. 2015), regardless of the infant's weight percentile (Unterscheider et al. 2013a).

In the long-term course, it is especially the neurodevelopmental disorders that shape the outcome (Torrance et al. 2010; Baschat 2011). According to a large Australian survey, children born near term with a birth weight <5th percentile have a 2.8-fold increased risk of infantile cerebral palsy compared to the reference group >10th percentile. This result was independent of the rate of asphyxia. If a fetal malformation was also present, the risk increased 30-fold (Blair and Nelson 2015). Similar data exist for the European region (Jarvis et al. 2003). It is possible that the risk of a neurological deficit is also increased if a cerebral perfusion disorder can be detected antenatally (Meher et al. 2015).

11.2.6 Diagnostics and Monitoring

A prerequisite for the correct diagnosis of SGA or FGR is knowledge of the gestational age. The sonographic measurement has the highest accuracy up to the 23rd week of pregnancy and is superior to the calculation of the date after the "confirmed last period." The measurement of the crown-rump length

in early pregnancy is currently the most reliable method for calculating the correct date of delivery (Butt et al. 2014).

If risk factors exist or clinical or sonographic assessments suggest the suspicion of FGR, ultrasound is also the preferred method for differentiating a pathological process. In principle, the diagnosis of FGR can only be made if a percentile drop can be observed in serial measurements of the fetal estimated weight. However, this approach is often not very practical in clinical reality. Therefore, an estimated weight <10th percentile is used as a diagnostic criterion and as surrogate for a reduced fetal growth velocity, even if this implies that a few FGR fetuses >10th percentile are not detected, and many constitutional SGA fetuses are wrongly attributed to a risk group. The most common formula for fetal weight estimation is that according to Hadlock et al. (1985) including head circumference, abdominal circumference and femur length. Because of the decreased liver growth typically affected early, measurement of abdominal circumference has the highest value.

For fetal weight estimation, the most important sonographically detectable biometric measures include: biparietal diameter, head circumference, abdominal circumference, femur length, and humerus length.

When interpreting fetal biometry, several factors must be considered:

- Estimation error of the ultrasound. A deviation <10% of the actual weight is achieved in up to 70% of cases at best. A sufficient time interval of at least 2 weeks should be observed to assess growth. The false positive rate decreased in one study from 31% to 17% and 3%, respectively, when measuring again after 1, 2, or 4 weeks (Mongelli et al. 1998).
- Symmetrical/asymmetrical FGR. In a study with 151 children, for example, a sensitivity of 73% for an FGR was achieved for the asymmetrical examination group, and 59% for the symmetrical group (Simon et al. 1990).
- Timing of the ultrasound measurement. The sensitivity of the measurement increases in late pregnancy. An optimal screening time seems to be between 32 and 36 weeks gestation (Roma et al. 2015).
- Abdominal circumference percentile cut-off. In a study of 2237 patients, an abdominal circumference <5th percentile in term infants and <10th percentile in preterm infants was associated with increased perinatal morbidity (Sheth and Glantz 2016).

A fetal estimated weight <10th percentile or abdominal circumference <10th percentile should prompt further diagnosis for FGR.

Especially in early (<24 weeks of gestation) and symmetrical SGA with polyhydramnios and unremarkable Doppler ultrasound, the findings should suggest fetal malformation or infection. This should prompt a malformation screening. If necessary, a fetal karyotyping/microarray examination or TORCH screening (screening for toxoplasmosis, rubella, cytomegalovirus, herpes simplex infection and other infections) should be performed.

Finally, a number of sonographic parameters can be added to further differentiate between constitutional SGA and FGR and allow risk assessment.

Growth Dynamics

Sonographic follow-up measurements of fetal biometry can confirm the diagnosis of FGR. Primarily, the growth dynamics of fetal estimated weight and abdominal circumference is observed (de Jong et al. 1999). Regardless of gestational age, the cut-off of the growth dynamics of abdominal circum-

ference is considered to show an increase of at least 10–14 mm within 2 weeks (Divon et al. 1986).

Head-Abdomen Discrepancy

Since fetal compensatory mechanisms aim to maintain nutritive supply to the head (brain-sparing effect), the majority of FGR result in an asymmetric appearance with only minor restriction of head circumference. Relating abdominal circumference to head circumference may further mitigate the false positive rate in SGA fetuses (Campbell and Thoms 1977). However, this is at the expense of sensitivity.

Amniotic Fluid Volume

As a result of the redistribution of circulating blood volume during FGR, the blood supply to the kidneys is reduced (Nicolaides et al. 1990). The resulting decrease in urine production consecutively leads to a smaller amount of amniotic fluid. Oligohydramnios has therefore been evaluated for the diagnosis of FGR and as a means of assessing fetal well-being. However, its value is limited by the fact that, on the one hand, other circumstances (premature rupture of membranes, congenital anomalies especially of the urogenital tract, transfer, etc.) can also cause oligohydramnios and, on the other hand, only a proportion of FGR fetuses actually have oligohydramnios. Thus, oligohydramnios is unsuitable as a screening parameter (Chauhan et al. 2007). In combination with a fetal estimated weight <3rd percentile, the oligohydramnios is predictive of fetal outcome (Unterscheider et al. 2013a).

Biophysical Profile

The simplest method of fetal monitoring is the monitoring of fetal activity by the mother. A subjectively perceived decrease in fetal activity by the mother is associated with an increased risk of fetal death or pathological CTG (Frøen et al. 2008).

The biophysical profile allows a standardized quantification of the fetal activity and can thus indicate the degree of risk. An examination period of 30 min is recommended for this purpose (Manning 2002).

Doppler Ultrasound

Doppler sonography provides information on maternal and fetal hemodynamics. Since this is directly dependent on placental function, abnormal flow patterns may indicate placental dysfunction. A measurable increase in the flow resistance of the umbilical arteries, as can be observed in particular in early onset FGR, occurs when the villous vessels are obliterated by 30% or more (Morrow et al. 1989). However, even in late onset FGR, histological features of reduced perfusion in the placenta can be associated with pathological flow patterns of certain vessels (umbilical vein, uterine artery), even if the flow patterns of the umbilical arteries are inconspicuous (Parra-Saavedra et al. 2014).

The most frequently examined fetal vessels are the umbilical arteries, the middle cerebral arteries, the ductus venosus and the umbilical vein.

Umbilical Arteries and Descending Aorta

Since the umbilical arteries are often the first vessels in which a pathological flow pattern can be detected, especially in severe early FGR before the 34th week of gestation, it was hoped that this could be used as screening tool. Indeed, the combination of a low estimated weight and increased resistance of the umbilical arteries increases the positive predictive value for FGR to 77% with a negative predictive value of 93% (Ott 1990). Progression of pathological Doppler values towards end-diastolic absent and further to reversed flow (ARED flow) represents an increasing deterioration of the fetal condition (Ferrazzi et al. 2002;

Kiserud et al. 2006; Turan et al. 2008; Unterscheider et al. 2013b).

Middle Cerebral Artery and Cerebroplacental Ratio (CPR)

A decrease in fetal arterial pO_2 leads to a decrease in the pulsatility index in the cerebral circulation (Arbeille et al. 1995). This brain-sparing effect was previously thought to be downstream of the high impedance of the umbilical arteries. This dogmatic progression of fetal circulatory dysfunction from placental to cerebral vessels could only be partially reconstructed on the basis of the PORTO study. Of 1116 SGA fetuses, 511 (46%) had increased resistance of the umbilical arteries, and 300 (27%) had decreased resistance of the middle cerebral artery. However, the combination of both was much less frequent than expected. 17% of fetuses with pathologic umbilical artery findings had or developed middle cerebral artery pathology. Conversely, 56% of fetuses with pathological middle cerebral artery findings had an unremarkable flow pattern of the umbilical artery (Unterscheider et al. 2013b). This seems to be particularly relevant in late onset FGR. The value of this late brain sparing may be further surpassed when the cerebroplacental ratio (CPR), i.e., the ratio of the pulsatility or resistance indices of the middle cerebral artery and the umbilical artery, is formed (DeVore 2015). In the PORTO study, the formation of the CPR increased specificity in predicting adverse fetal outcome (OR 11.7; 95% CI: 6.0–22.9), however, at the expense of sensitivity when this approach was compared to the value of a pathologic umbilical artery finding (OR 6.9; 95% CI: 2.9–16.5) (Flood et al. 2014).

Ductus Venosus

If an ARED flow pattern of the umbilical artery subsequently occurs, the likelihood of pathological venous flow patterns increases. The clinical latency period between arterial and venous pathology is between four and six weeks (Turan et al. 2008). It is initially noticeable in an increase in the pulsatility index for veins in the ductus venosus. In the course, this can then lead to absent or reversed flow during atrial systole in the ductus venosus (so-called negation of the a-wave) as well as multiphasic pulsations in the umbilical vein (Hofstaetter et al. 2002; Haugen et al. 2004; Kiserud et al. 2006).

In early FGR, the focus is on the umbilical arteries and venous Doppler parameters (ductus venosus), whereas in late FGR, the Doppler of the middle cerebral artery or CPR better reflects clinical progression (Fig. 11.5).

11.2.7 Therapeutic Management–Delivery Indication

The aim of obstetric management is primarily to determine the optimal date of delivery. In general, the aim is to avoid intrauterine fetal death or specific sequelae of severe (acute) intrauterine hypoxia on the one hand, and to prolong the pregnancy on the other hand in order to minimize premature birth-associated mortality and morbidity. Therefore, the further procedure is strongly dependent on the individual prerequisites of the pregnant woman. Here, the gestational age plays a decisive role, as does the assessment of the acute risk by Doppler parameters and cardiotocography (CTG), as well as risk factors of placental insufficiency. The expectant parents should be closely involved in this decision-making process. Especially at the borderline of viability, prolongation of pregnancy to improve neonatal outcome may be an option in cases of severe fetal impairment, even at the risk of intrauterine fetal death. So-called PREM scores can help in counseling parents (Tyson et al. 2008). However, perinatal centers should also be able to use their own data as a basis.

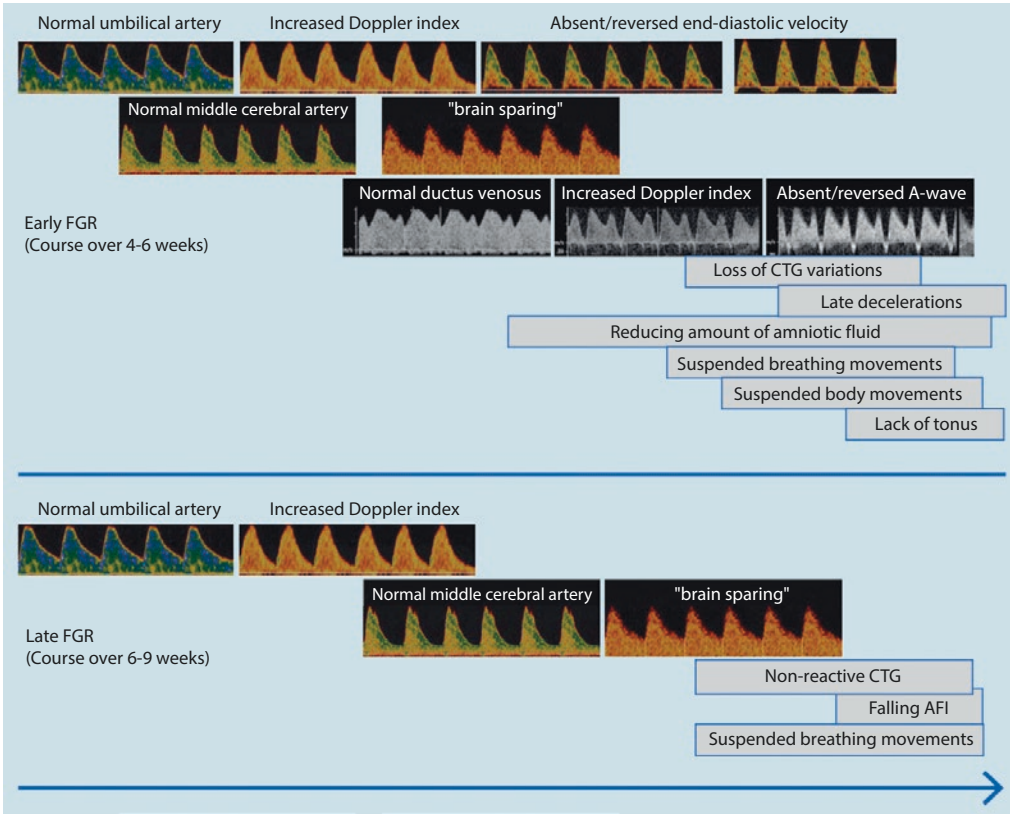


Fig. 11.5 Clinical progression in early and late FGR. In early onset FGR, there may be a rapid or somewhat slower progression, depending on placental blood flow resistance. Arterial and venous Doppler changes often occur sequentially and are followed by CTG changes and a reduction in body movements. In contrast, late onset FGR progresses more slowly. Changes in Doppler parameters are often much less pronounced or undetectable. (From Baschat 2013)

Tip

The National Institute of Child Health and Human Development (NICHD) provides an online outcome calculator (survival and survival without neurologic deficit) for very preterm birth (22 and 25 weeks gestation). (► https://www.nichd.nih.gov/about/org/der/branches/ppb/programs/epbo/pages/epbo_case.aspx; accessed 11/28/2017).

Between weeks 26 and 29, each day in utero provides about 1–2% survival advantage (Baschat et al. 2007). If a preterm birth is expected, the preparation of the fetus follows the general rules including induction of lung maturity with corticoids and neuroprotection with magnesium sulphate.

Up to about 34 weeks gestation, Doppler ultrasound of the umbilical arteries is the most important method of assessing risk and monitoring the fetus. If the flow pattern

is inconspicuous, it is sufficient to check the findings every 1–2 weeks. Increased resistances (resistance index or pulsatility index >95th percentile) give reason for closer monitoring including other vessels (middle cerebral artery, ductus venosus), although little value is attributed to the middle cerebral artery or CRP before 32 weeks gestation in relation to the indication for delivery (Stampalija et al. 2017). If progression to ARED flow occurs, a 1- to 3-day repeated measurement is reasonable. The absence of positive end-diastolic flow precedes abnormal CTG patterns by about a week on average (Arabin et al. 1988). Daily CTG monitoring, preferably with computerized evaluation of the short-term variation (Oxford CTG) is therefore necessary. Delivery in the case of end-diastolic reversed flow in the umbilical arteries is indicated at the latest at 32 weeks gestation, and at 34 weeks gestation in the case of absent flow. It is controversially discussed whether changes in the short term variation or the ductus venosus are more appropriate as additive parameters in this FGR group (Visser et al. 2016).

If the end-diastolic flow profile of the umbilical artery is positive and other parameters are otherwise normal, the pregnancy can be prolonged until close to term under close monitoring. If there are additional risk factors or signs of placental insufficiency, such as oligohydramnios, fetal growth arrest, preeclampsia or CTG changes, premature delivery is indicated. However, the data on delivery criteria after 34 weeks gestation is sparse. The value of CPR after 34 weeks is currently under scrutiny.

Three large multicenter randomized intervention trials have been conducted with the aim of determining the appropriate time of delivery:

Growth Restriction Intervention Trial (GRIT)

Although the title suggests FGR, there was no agreed definition of this in the GRIT study. Ultimately, 587 women between 24 and 36 weeks gestation were included in whom the obstetrician was unsure whether it was better to terminate the pregnancy because he or she expected fetal death or a neurological complication on prolongation. In 40%, the fetus had an ARED flow pattern. Following randomized procedures, 296 women were delivered directly. In 291 women, the pregnancy was prolonged for as long as seemed maximally justifiable by the obstetrician. There were no other intervention criteria. The primary endpoint was survival without neurological deficit after 2 years.

The mean prolongation of pregnancy was 4.9 days in the corresponding study arm. There were fewer stillbirths in the delivery group (two vs. nine in the prolongation group), but a higher postnatal mortality (27 vs. 18). In terms of neurological deficits, there was no significant difference between the two study arms after up to 13 years. However, there was a trend towards a higher rate of cerebral palsy in the children of the delivery group (5% vs. 1% in the prolongation group). Therefore, at least for preterm deliveries <32 weeks of gestation, the data refute the theory that prolongation in an adverse (hypoxic) uterine environment would cause neurological damage (Thornton et al. 2004; Walker et al. 2011).

Disproportionate Intrauterine Growth Intervention Trial at Term (DIGITAT)

Included were 650 pregnant women after completion of the 36th week of gestation with a fetal estimated weight or abdominal circumference <10th percentile or evidence

of a drop from the growth percentile. Intervention consisted of immediate induction of labor (321 women) or prolongation with close monitoring until the onset of spontaneous delivery or other delivery indications (329 women). The primary endpoint was a composite of various parameters of neonatal morbidity and mortality.

In the prolongation group, delivery was on average 10 days later and newborns weighed on average 130 g more. There was no significant difference in the neonatal composite outcome (5.3% vs. 6.1% in the delivery group) or the rate of C-sections (approx. 14%). Also in the follow-up after 2 years, no difference in child development between the two groups could be detected. However, it must be mentioned restrictively that women with lack of fetal movements or suspicious CTG changes were excluded from the study. In addition, only about 30% of the prolonged pregnancies showed a further decline in the growth curve. Therefore, it can be assumed that a large proportion (70%) of constitutional SGA fetuses positively influenced the outcome (Boers et al. 2010; van Wyk et al. 2012).

Trial of Randomized Umbilical and Fetal Flow in Europe (TRUFFLE)

The inclusion criteria of the TRUFFLE study consisted of pregnant women between 26 and 32 weeks gestation with fetal abdominal circumference <5th percentile and increased resistance of the umbilical artery >95th percentile. 503 women were divided into three groups. The CTG group (166 women) was delivered at a short-term variation in Oxford CTG <3.5 ms (<29 weeks of gestation) or <4.0 ms (≥29 weeks of gestation). In a second group (167 women), delivery was performed when early changes in the ductus venosus occurred (PIV >95th percentile). In the third group (170 women), delivery was indicated if the ductus venosus showed late changes in terms of absent or reversed flow (negative a-wave). The primary outcome was defined as survival without neurological deficit at 2 years. In the end, the group of children delivered on the basis of the short-term variation showed a lower perinatal mortality but a less favorable long-term neurological outcome compared to the group delivered after the absence of a-wave in the ductus venosus. However, we are talking about only a few percentage points difference between the groups, because the overall results both in terms of perinatal mortality (8%) and survival without neurological deficit (90%) were encouragingly good in all groups despite the high-risk population. This may also be due to the fact that in many cases (51%) the indication for delivery was based on the narrowly defined safety net (11%) (short-term variation <2.6 ms [<29 weeks of gestation], or <3.0 ms [≥29 weeks of gestation]) or concomitant maternal circumstances such as preeclampsia (11%), or delivery was also scheduled after 32 weeks of gestation (29%). There was also no difference in neonatal composite outcome between the groups. 69% of the children survived without severe neonatal morbidity (Lees et al. 2013, 2015).

A clear recommendation for action cannot be derived from any of these three studies. And so, despite all the evidence, the obstetrician also needs to have a gut feeling.

11.3 Preeclampsia: Diagnosis and Management

Holger Stepan

Preeclampsia is one of the most common complications of pregnancy. It affects about 2–3% of all pregnancies. The incidence of early onset preeclampsia, which manifests before the 34th week of pregnancy, is about 1% in Central Europe (Chaiworapongsa et al. 2014; Mol et al. 2016). For a long time, preeclampsia was considered a "disease of

theories," which has changed with the identification of angiogenic proteins as very important pathobiological factors. Now it is clear that in fact the placenta is the central "sick" organ and from it the crucial pathophysiological changes in preeclampsia, but also in HELLP syndrome and early fetal growth restriction (FGR) originate. Since, to date, delivery is the only causal treatment, preeclampsia causes a high proportion of iatrogenic preterm birth with all its medical and socioeconomic consequences (AWMF 2014; Schnettler et al. 2013; Roberts et al. 2003; Verlohren and Dudenhausen 2009). Furthermore, pregnancy with preeclampsia is a very relevant risk factor for later cardiovascular disease in affected women.

11.3.1 Definition and Classification

Preeclampsia is defined as the new onset of hypertension (≥140/≥90 mmHg) and proteinuria (≥300 mg/24 h) in the second half of pregnancy (>20th week). Other hypertensive pregnancy disorders such as gestational hypertension (synonym: pregnancy-induced hypertension, PIH), chronic or preexisting hypertension, and superimposed gestosis must be differentiated from preeclampsia. Gestational hypertension is when there is a new onset of hypertension without detectable proteinuria. Chronic hypertension is hypertension that was present before pregnancy or developed in early pregnancy. If patients with pre-existing hypertension also develop proteinuria during pregnancy, or if hypertension newly develops in patients with pre-existing proteinuria, this is referred to as superimposed gestosis (AWMF 2014). However, the definition and classification of hypertensive pregnancy diseases based on the clinical parameters of blood pressure and proteinuria will have to be modified in the future to include biomarkers.

11.3.2 Causes and Risk Factors

The causes of preeclampsia, which is also known as EPH gestosis, toxemia or pregnancy poisoning, are not fully understood. However, it is certain that a malfunction of the placenta is at the center of the disease. This is not new, as it has long been known that a placenta (but not a fetus) must be present for preeclampsia to develop, and that removal of the placenta is usually the only cure. A crucial role in the development of disease in a proportion of affected pregnant women is attributed to inadequate invasion of the trophoblast and subsequent inadequate transformation of uteroplacental blood vessels and glands (Goldman-Wohl and Yagel 2002). This process of pseudovasculogenesis is impaired in preeclampsia, probably resulting in hypoxemia/ischemia of the placenta (Zhou et al. 1997). The uteroplacental vascular territory retains a high resistance, whereas in a healthy pregnancy it is converted into a low-pressure system. In the meantime, however, it has become clear that the factor "disturbed trophoblast invasion" can be made responsible mainly for the early manifesting cases and is also observed in early FGR. For the diseases manifesting later or near term, other causal factors must be held responsible. Here, the focus is assumed to be more on the maternal side and less on the placental side. A maternal problem of the endothelium is discussed and it is suspected that women with a corresponding disposition develop preeclampsia more frequently and that pregnancy as a cardiometabolic stress test only unmasks the (latent) endothelial dysfunction.

One consequence of the placental defect is the release of placental factors into the maternal circulation (Lam et al. 2005). These factors may be the trigger or cause of generalized endothelial dysfunction and thus the maternal syndrome, including renal, car-

diovascular and neurological defects. In recent years, two of these factors have been identified as significant, although not alone explaining the disease. These are the angiogenic factors PlGF (placental growth factor) and sFlt-1 (soluble Fms-like tyrosine kinase-1). sFlt-1 is a soluble fragment of a VEGF (vascular endothelial growth factor) receptor. The angiogenic factor PlGF stimulates vascularization. In preeclampsia, the anti-angiogenic factor sFlt-1 is produced in excess. Its strong binding affinity to the pro-angiogenic PlGF results in a decrease of the free PlGF concentration (Hagmann et al. 2012; Levine et al. 2004). This imbalance between angiogenic and antiangiogenic factors is a major pathobiological phenomenon in the development of preeclampsia (Fig. 11.6). However, this placental pathology is not absolutely specific to preeclampsia but is also observed in other (related) clinical manifestations of placental dysfunction such as HELLP syndrome, FGR, premature placental abruption, or intrauterine fetal death. PlGF increases during the first two trimesters of normal pregnancy and decreases towards the end. In contrast, the concentration of sFlt-1 remains constant and increases only at the end. In women with early preeclampsia, decreased PlGF and increased sFlt-1 levels are measured (Levine et al. 2006). Other placental factors, such as soluble endoglin (sEndoglin), may have synergistic effects or additional pathogenetic significance in the development of preeclampsia (Levine et al. 2006; Dechend et al. 2004; Stepan et al. 2008; Cruz et al. 2012).

11.3.3 Diagnosis and Early Detection

The correct diagnosis is still a challenge, not least because the course of preeclampsia can be very variable. There are both relatively stable and mild forms, as well as acute onset forms with drastic clinical deterioration. The clinical overlap with the HELLP syndrome, which is regarded as a special manifestation of preeclampsia, but also with early FGR is large. The leading symptoms hypertension and proteinuria are non-specific and have a very low prognostic value. In order to prevent a premature and unjustified

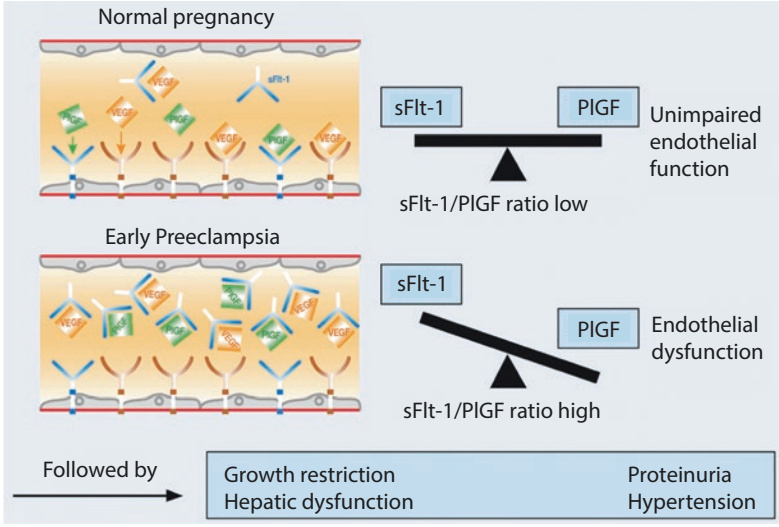


Fig. 11.6 Schematic representation of the pathogenesis of early preeclampsia. (Modified according to Hagmann et al. 2012)

termination of pregnancy on the one hand, but on the other hand to identify patients at high risk and to minimize the risk for mother and child, the differential diagnostic clarification, which is not trivial in clinical routine, is extremely important.

In the past, the measurement of blood pressure, the determination of the amount of protein in the urine and the anamnestic survey of risk factors were the focus of diagnostics. Doppler sonography of uterine arteries in the first and second trimester supports the diagnosis of preeclampsia. Nevertheless, differentiating preeclampsia from other forms of hypertensive pregnancy disorders can be difficult because other conditions, such as lupus erythematosus, can generate a preeclampsia-like phenotype. After 20 weeks of gestation, the leading symptoms of hypertension and proteinuria may indicate preeclampsia. Other possible signs include sudden severe weight gain, headache, malaise, upper abdominal pain, edema, and flickering vision (AWMF 2014). However, all of these symptoms are nonspecific and can occur without a preeclamptic background. Gestational hypertension, i.e. blood pressure values ≥140/90 mmHg without proteinuria in a previously normotensive pregnant woman, does not necessarily lead to preeclampsia (Verlohren et al. 2010). Atypical and relatively asymptomatic courses also occur. In addition, blood pressure and protein measurements in particular are not very accurate and are prone to error (Hagmann et al. 2012), and in many critical cases the leading symptoms are absent altogether. Early diagnosis and assessment of the dynamics of preeclampsia before the onset of threatening symptoms is not always possible in time with standardized blood pressure and urine diagnostics, but is still essential and standard today for the diagnosis and classification of the disease. To date, the only screening method for the detection of preeclampsia that is firmly anchored in clinical routine is the Doppler sonographic examination of the uterine blood vessels as part of second trimester screening. However, the sensitivity and specificity of the examination method is limited (Zeisler et al. 2016). The negative predictive value especially of the uterine Doppler in the first trimester is very good, which can be of great benefit especially for pregnant women with a burdened anamnesis.

11.3.4 Biomarkers in Diagnostics and Prediction

The current concept for the development of preeclampsia postulates a dysbalance between anti-angiogenic factors (sFlt-1) and the angiogenic ligands of the VEGF receptor such as PlGF or VEGF. By blocking angiogenesis and thus damaging the endothelium, global endothelial dysfunction ultimately occurs, which is responsible for the symptoms of preeclampsia—hypertension, proteinuria (Dechend et al. 2004; Stepan et al. 2008; Cruz et al. 2012). In recent years, placental angiogenic factors PlGF and sFlt-1 have been evaluated for daily use in the clinic. In a study of pregnant women, it was shown that sFlt-1 concentration is usually elevated and PlGF concentration is usually decreased in early preeclampsia. Calculation of the sFlt-1/PlGF ratio allows reliable differentiation of pregnancies with and without early preeclampsia (Fig. 11.7) (Verlohren et al. 2010).

With the preeclampsia markers PlGF and sFlt-1, two biomarkers are available for clinical routine that effectively facilitate and accelerate the diagnosis of early onset preeclampsia. A cutoff value of 85 for the ratio of sFlt-1 and PlGF resulted in a clinical sensitivity of 82% with a clinical specificity of 95% (Verlohren et al. 2010; Hagmann et al. 2012). Diagnostic accuracy for early onset preeclampsia <34 weeks of gestation is high, with a sensitivity of 89% and a specificity of 97%. Prediction in the at-risk population is

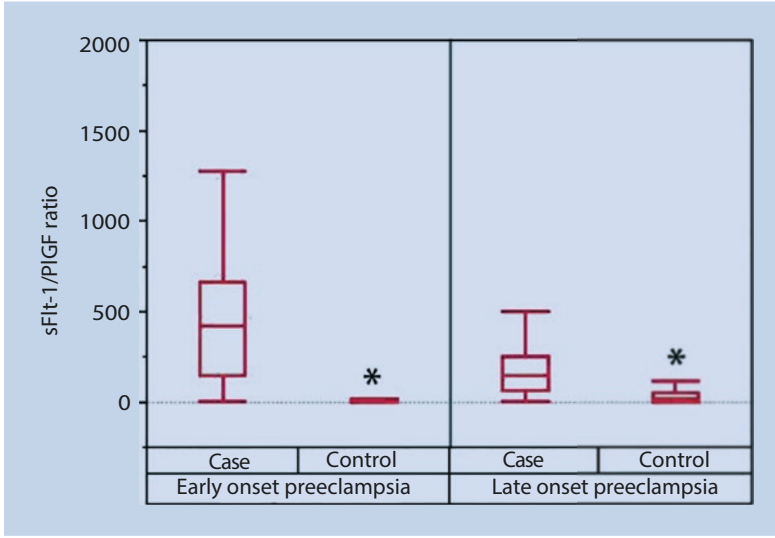


Fig. 11.7 sFlt-1/PIGF ratio <34th week of gestation vs. >34th week of gestation (box/whisker plot). (According to Verlohren et al. 2010)

also very good. General screening, on the other hand, is not useful. A low sFlt-1/PlGF ratio can exclude the non-occurrence of preeclampsia, eclampsia or HELLP syndrome for 4 weeks with a negative predictive value of 94.3%. A high ratio (>38) can predict the occurrence of a maternal and/or fetal complication within 4 weeks with a positive predictive value of 65.5% (Verlohren et al. 2012). Relevant for a better treatment outcome is also the clarification and monitoring of clinically unclear situations. In severe preeclampsia or HELLP syndrome, markers allow short-term prognosis, i.e. an estimate of how much time is likely to be left before delivery is necessary. At sFlt-1/PlGF ratios of >655 before completed 34 weeks of gestation or >201 after completed 34 weeks of gestation, delivery is very likely to be required within 48 h (Poon et al. 2009). The inverse relationship between the level of the sFlt-1/PlGF ratio and the time remaining from diagnosis or measurement to delivery has been confirmed in several studies. Interestingly, this relationship is also observed in pregnant women who do not develop clinical preeclampsia, so that an elevated sFlt-1/PlGF ratio also indicates an increased risk of preterm delivery independent of the classical diagnostic criteria.

11.3.5 Risk Assessment for Preeclampsia in the Context of First Trimester Screening and Secondary Prophylaxis

An algorithm for the risk assessment of preeclampsia in the context of first trimester screening is available for clinical use. With its high negative predictive value, this can predict with good certainty an inconspicuous course of pregnancy in the case of an inconspicuous finding, but can also identify patients at risk who will benefit from secondary prevention such as acetylsalicylic acid (ASA). The concept is now to couple early preeclampsia diagnosis with first trimester screening in terms of the "inverted pyramid of antenatal care." This seems to be

useful and reasonable insofar as the risk assessment can be made very early, which in turn allows to exploit the positive effect of ASA accordingly and to integrate the pregnant woman into an appropriate care concept very early. Since a blood sample is taken anyway during first trimester screening and the perfusion of the uterine arteries can also be measured by Doppler sonography, it makes sense to determine biomarkers at this time. Initially, it was observed that pregnancies with low PAPP-A values ("pregnancy associated plasma protein A") in the first trimester screening, but with a normal karyotype, more frequently developed preeclampsia or FGR in the further course of pregnancy. In 2009, it was shown that the risk of subsequent preeclampsia increases significantly from a MoM <0.4 of the PAPP-A value. There are now algorithms for calculating the risk of preeclampsia using the PAPP-A and PlGF values in combination with uterine perfusion values and maternal blood pressure. In a 2011 study, it was demonstrated that a combination of maternal factors such as uterine Doppler and blood pressure in combination with a set of the above markers is able to detect early onset preeclampsia with a detection rate of 91% and late onset preeclampsia with a detection rate of 60.9% at a false positive rate of 5% (Akolekar et al. 2011). The good negative prediction of the test is particularly useful (e.g., in "condition after preeclampsia") (Rolnik et al. 2017). In case of a "positive" test, i.e. the detection of a probably increased risk of preeclampsia (>1:100), the situation is more difficult and the counselling situation more complex. Ultimately, a relevant number of pregnant women are also overclassified and potentially unsettled because, in contrast to aneuploidy screening, there is no immediate further diagnosis available and intervention options are limited. However, it has now been shown in a large study that daily administration of 150 mg ASA highly significantly reduces the likelihood of subsequent preeclampsia, mainly reducing cases with early onset of the disease (Ferrer et al. 2000). Alternative options for secondary and tertiary prevention such as heparin, magnesium, calcium, zinc, selenium, fish oil and others have no proven effect.

11.3.6 Clinical Management

Despite progress in research into the etiology, no causal therapy exists to date. Drug therapy is always only a symptomatic treatment of the functional limitations of the maternal organism. Such therapy has as its primary goal, in addition to the avoidance of maternal blood pressure peaks, the prolongation of pregnancy in order to avoid or delay a premature birth with adverse consequences for the perinatal outcome. Such conservative management is indicated between 23 and 34 weeks of gestation. The primary goal in this situation is lung maturation induction in the fetus through the administration of glucocorticoids and monitoring in the perinatal center. On average, a prolongation of 5–10 days can be achieved, which has a decisive influence on the fetuses' chances of survival, especially between 24 and 26 weeks of gestation. Indications for termination of pregnancy are hypertensive crises refractory to therapy, persistent central nervous symptoms in the form of headache, vomiting or flickering vision, increasing oligo- or anuria, falling platelet counts or derailing coagulation parameters in HELLP syndrome or signs of fetal distress (pathological CTG). After 34 weeks of gestation, pregnancy should be terminated in any case of proven preeclampsia or HELLP syndrome. The mode of delivery must be chosen according to the clinical condition of mother and child, but does not exclude vaginal delivery (AWMF 2014).

A benefit for the fetal development and thus an improvement of the fetal prognosis

by a medicinal reduction of blood pressure could not be proven so far. Therefore, the main focus of therapy is to avoid maternal cerebro- and cardiovascular complications. Antihypertensive therapy should be started at blood pressure values ≥170/110 mmHg, and in the case of preexisting hypertension or clot constellation already from blood pressure values of ≥160/100 mmHg (Helewa et al. 1997). The goal of drug therapy is to reduce blood pressure to values between 140–160/90–100 mmHg. Due to the maternal risk, untreated blood pressure values between 160–170 mmHg systolic and 100–110 mmHg diastolic can only be tolerated for a short time, asymptomatically and only under inpatient observation, as the cerebral vascular system is not yet adapted to high blood pressure values, particularly in preeclampsia, and a break in cerebrovascular autoregulation with a risk of cerebral hyperperfusion cannot be ruled out. Various antihypertensive drugs can be considered as drug therapy. For long-term control, methyldopa, which acts centrally on alpha receptors, is the drug of choice. The second choice is a cardioselective beta-blocker, which should not be used in cases of fetal growth restriction, as recent meta-analyses describe lower birth weights under beta-blocker therapy. For acute blood pressure lowering, the calcium channel blocker nifedipine can be administered orally. For intravenous therapy, the alpha receptor antagonist urapidil is available. Magnesium is used as a vasorelaxant drug in the prophylaxis and therapy of eclampsia (Lucas et al. 1995; Woudstra et al. 2010). By decreasing cerebral vascular resistance, cerebral blood flow is increased and the risk of eclampsia is decreased. Overdose can lead to respiratory depression, so respiratory rate (>12/min), oxygen saturation, and patellar tendon reflex should be monitored as parameters of cerebral depression during intravenous magnesium administration. The therapeutic range of serum magnesium level is between 2–4 mmol/l. Calcium gluconate is available as an antidote.

The significance of glucocorticoid administration in HELLP syndrome and a platelet count <100,000/µl has not yet been conclusively clarified. Some studies show a faster recovery of platelets and an improvement of the clinical condition compared to placebo administration, other studies could not confirm these results (Thadhani et al. 2011).

11.3.7 Possible Future Therapeutic Approaches

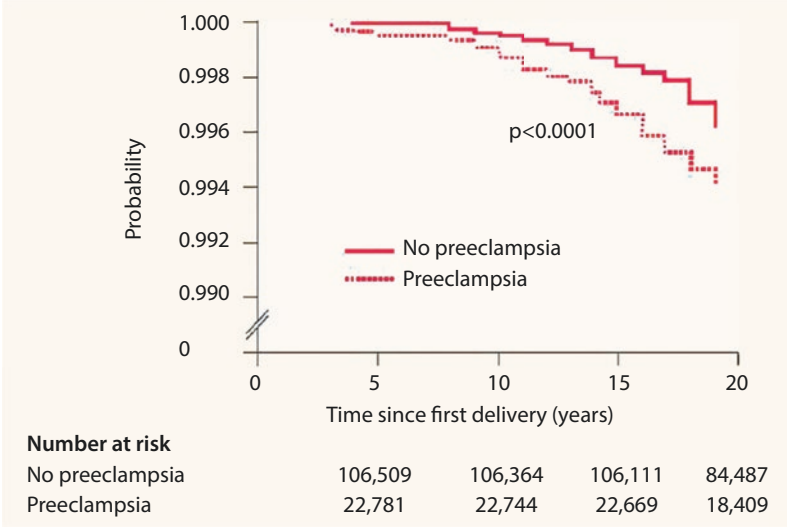
Although no real treatment options exist today other than delivery, there are some hopeful approaches that are currently being pursued and tested in studies. These therapeutic options aim at reducing maternal symptoms, not affecting the fetal situation and allowing prolongation of pregnancy via stabilization of the clinical situation. The angiogenic imbalance is a possible target. sFlt-1-blocking antibodies are too risky due to placental permeability and therefore not a promising approach in humans. An alternative may be filtering procedures that extract the causative agent sFlt-1 from the maternal circulation, especially since extracorporeal apheresis has already been used for other indications in pregnancy (Thadhani et al. 2016). Pilot studies provided first promising results in prolonging pregnancies of preeclamptic women by apheresis. Here, one exploits the fact that sFlt-1 carries a positive charge at physiological pH. Negatively charged dextran sulfates of apheresis columns (Thadhani et al. 2016; Lefkou et al. 2016) bind these same positively charged domains. A reduction in sFlt-1 level of 25–30% in one time adsorption vs. 17–34% in multiple adsorptions, an improvement in the protein/creatinine ratio, and also a stabilization of blood pressure by minimizing proteinuria could be achieved. Alternatively, in animal models, PlGF infusion was also

shown to positively affect the angiogenic balance, which also resulted in a reduction in blood pressure and decrease in proteinuria induced in the model. Pharmacologically, statins currently allow a promising approach. Pravastatin, as a 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor, also affects the angiogenic balance by decreasing sFlt-1. Initial pilot studies in humans (pregnant women at high risk for preeclampsia or antiphospholipid syndrome) appear to benefit clinically from the pleiotropic effects of statins (Costantine et al. 2016; Smith et al. 2001). Whether statins can really improve clinical outcome in preeclamptic pregnancies remains to be tested in randomized controlled trials.

11.3.8 Long-Term Morbidity

The view of preeclampsia is changing from a purely obstetric problem to a more internal medicine consideration, because a pregnancy with preeclampsia is the cardiovascular risk factor par excellence for a woman.

A study published in the Lancet in 2001 showed that long-term survival 10–20 years after pregnancy was worse in women who had preeclampsia compared with women who had a normal pregnancy (◘ Fig. 11.8) (Garovic and Hayman 2007). There are now a large number of studies demonstrating that the occurrence of preeclampsia in pregnancy significantly increases the relative risk of chronic ischemic heart disease, hypertension or myocardial infarction in the future (Craici et al. 2008; Rodie et al. 2004). In this regard, the likelihood of cardiovascular disease or cardiovascular-related death appears to be highest when the pregnancy was firstly complicated with preeclampsia, secondly ended or had to be terminated as a preterm delivery, and thirdly when a growth-restricted child was born (Garovic and Hayman 2007). Based on these convincing epidemiological relations, it can be concluded that preeclampsia is the first early expression of a lifelong risk predisposition for cardiovascular disease or the metabolic syndrome. In a healthy woman, pregnancy as a cardiometabolic stress test does not exceed a threshold of



◘ **Fig. 11.8** The long-term survival of women who have had preeclampsia is worse than that of women with normal pregnancies. (Adapted from Smith et al. 2001)

clinical symptoms and may not reveal internal disease until later in life and with the addition of other lifestyle factors (obesity, smoking) (Girouard et al. 2007). In contrast, women with a generally increased cardiovascular risk already cross this threshold during pregnancy and manifest preeclampsia there. After pregnancy, these women initially become clinically asymptomatic again by falling below the threshold again, but manifest cardiovascular/cardiometabolic disease much earlier in progressive life. Metabolic changes can persist for a very long time after preeclampsia. For example, a long-term study demonstrated that increased insulin resistance is detectable 8 years after preeclampsia. Cardioprotective adipocytokines such as adiponectin are decreased, while homocysteine, leptin, insulin and apo-B/apo-A1 rates are increased (Vikse et al. 2008). A large Scandinavian cohort study demonstrated that women who have had a hypertensive pregnancy complication have a significantly higher risk of end-stage renal failure with consecutive need for dialysis and renal transplantation in old age compared to women who had a healthy pregnancy or no pregnancy at all (Thadhani and Solomon 2008). Interestingly, the risk was highest in women who had multiple pregnancies with hypertensive complications. Although many questions are currently unanswered with regard to issues such as causality or primum of pathological changes, it seems useful to further investigate cardiometabolic stress indicators in preeclamptic pregnant women. Since internal medicine research of metabolic syndrome as a common and civilization disease is intense, obstetric research in the field of preeclampsia can benefit from it. This relatively new view on hypertensive pregnancy complications requires not only leaving the "time scale" as a pure (time-limited) disease of pregnancy, but also a long-term and internistically oriented follow-up as well as follow-up of the affected women.

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The Placenta in Twins

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Contents

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12.1 Introduction

The management of a twin pregnancy starts with the correct assessment of the chorionic and amniotic conditions already at the first trimester ultrasound. There are numerous differences between singleton and twin placentas. While dichorial placentas may resemble those of singletons, monochorial placentas are fundamentally different. Understanding the architecture of twin placentas is critical to understanding the mechanisms of pathologies.

In this chapter, the differences between monochorial and dichorial placentas are explained in more detail. Furthermore, the significance and pathology of the placenta in twin-to-twin transfusion syndrome and discordant twin growth is explained.

12.2 Structural Differences Between Monochorial and Dichorial Placentas

12.2.1 Chorionicity

Monochorial (MC) and dichorial (DC) twins are defined by the structural differences of their placentas. Although the majority of DC placentas are fused, two completely separate placentas are found in 44%. In contrast, MC placentas are usually composed of a single placental mass. Only rarely bipartite monochorial placentas are observed (Zhao et al. 2016). The separating membrane of MC twins is thinner and consists of only two amniotic layers, whereas in DC twins a third layer, the chorion, lies between these amniotic membranes.

The main difference between an MC and DC placenta is the presence or absence of vascular anastomoses. Nearly all (94–99%) MC placentas have vascular anastomoses, whereas these are not observed in DC placentas (Zhao et al. 2016; Denbow et al. 2000; Lewi et al. 2007) (Fig. 12.1). Anastomoses are visible on the chorionic plate. Three types of vascular anastomoses are distinguished: arterioarterial (AA), venovenous (VV), and arteriovenous (AV). AA and VV anastomoses consist of two arteries and two veins, respectively (one from each twin), which connect directly on the chorionic plate and are therefore referred to as "superficial." They are bidirectional, meaning that the direction of blood flow depends on the intraplacental pressure gradient. In contrast, AV anastomoses are always unidirectional, meaning that blood flow runs from the artery of one twin to the vein of the other. They do not connect directly on the placental surface but in the depth of divided cotyledons, hence the name of "deep" anastomoses. On the placental surface, AV anastomoses appear as a separate artery and vein of each twin, meeting on the chorionic plate and then dipping side by side into the MC placenta (Lewi et al. 2013). AA anastomoses are observed in 87% of monochorial diamniotic (MCDA) twin placentas, whereas VV anastomoses occur in only 25% of cases.

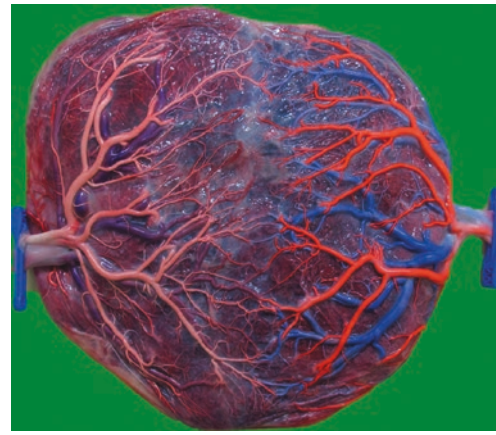

Fig. 12.1 Monochorial diamniotic placenta from an uncomplicated twin pregnancy delivered at 36 weeks gestation. The placenta is equally distributed and there are numerous vascular anastomoses between the twins

Both forms usually occur solitarily, and clustered occurrence in a placenta is rare. In contrast, AV anastomoses are present in 94% of MCDA placentas and rarely occur alone. In most cases AV anastomoses occur together with AA or/and VV anastomoses. Solitary AV anastomoses are present in only 5% of MCDA placentas (Lewi et al. 2007). In monochorial monoamniotic (MCMA) placentas, AA anastomoses are present in almost all cases (Umur et al. 2003). AA anastomoses can be visualized by Doppler ultrasonography, especially in anterior wall placentas, with a sensitivity of 75%. They show a typical bidirectional flow pattern of pulse waves (Fichera et al. 2005; Hecher et al. 1994). The unidirectional AV anastomoses may result in unbalanced blood flow from one twin to the other. However, because AA anastomoses are bidirectional, they can act as a "flexible AV anastomosis" to compensate for any imbalance (Lewi et al. 2013). Although VV anastomoses are also bidirectional, they do not appear to have this protective effect.

12.2.2 Assessment of the Placenta in Early Pregnancy

Chorionic and amniotic relations can be determined by counting the number of membranes between twins. Many textbooks recommend using the so-called lambda sign and the T-sign to distinguish dichorial from monochorial diamniotic (MCDA) twins. This can lead to confusion because the T-sign is not detectable in all monochorial twins in the first trimester. Most commonly, one observes an "empty lambda sign" resulting from apposition of two amniotic cavities with the uterine wall (Fig. 12.2a). While in an MCDA situation only two amniotic membranes separate the twins, in a DC pair the twins are separated by three layers: the two thin amniotic membranes and an intervening thick chorionic layer. This situation is referred to as the "full lambda sign" or "twin-peak sign," which results from hyperechogenic chorionic tissue between the two amniotic membranes (Fig. 12.2b) (Lewi et al. 2010). In monochorial monoamniotic (MCMA) twins, there

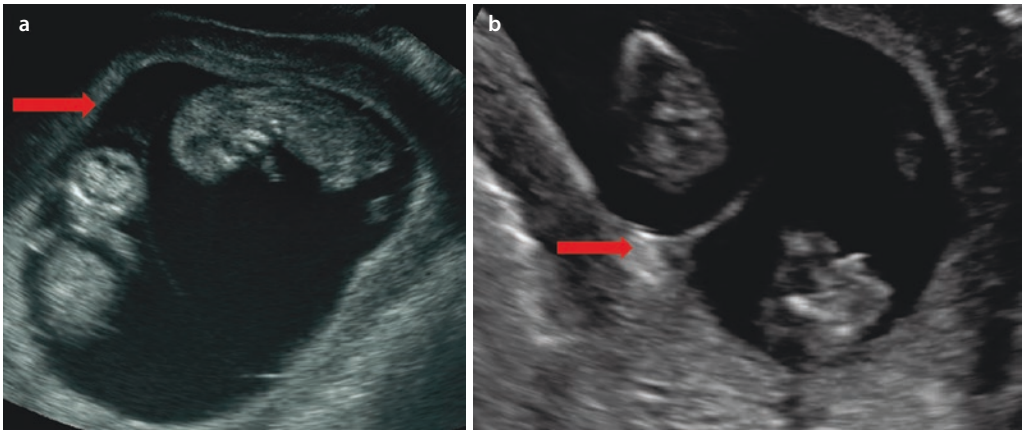

Fig. 12.2 **a**, **b** Lambda sign. **a** So-called empty lambda sign, as usually present in an MCDA twin pregnancy instead of a T-sign. Unlike the full lambda sign in DCDA twins, there is no chorionic layer between the two amniotic membranes. **b** Typical ultrasound image of the full lambda or twin-peak sign (*arrow*) in an early DC twin pregnancy. The broad echogenic chorion is wedged between the amniotic membranes

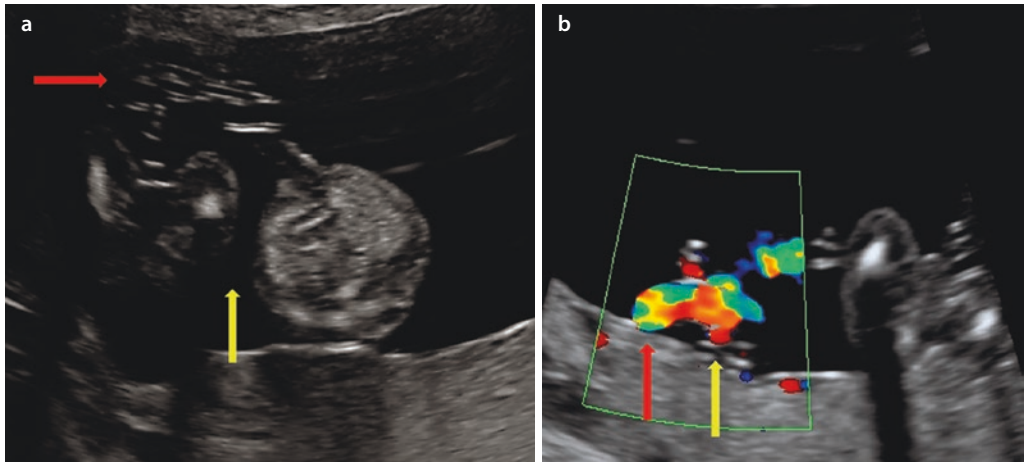

Fig. 12.3 **a**, **b** MCMA twins. **a** Ultrasound image of an MCMA twin pregnancy. There is no intertwin membrane (*yellow arrow*) and the umbilical cords are tangled together (*red arrow*). **b** Doppler image of the umbilical cords in an MCMA pregnancy. The arrows indicate the umbilical cord insertions that are close together. The umbilical cords are tangled together

is no separating membrane, and the fetuses lie in the same amniotic cavity. The cord entanglements that typically occur in this case can be detected as early as the first trimester using color and/or pulsed wave Doppler ultrasound (Fig. 12.3a, b) (Sherer et al. 2002).

In rare cases, the chorionicity of a pregnancy is not clear. The placenta may be partially dichorial and partially monochorial (Galjaard et al. 2014; Murata et al. 2016). In general, such pregnancies should be managed as monochorial pregnancies because of the presence of vascular anastomoses. There are isolated case reports of a so-called false lambda sign, which can lead to the misdiagnosis of dichorionicity in a twin MC pregnancy (Gueneuc et al. 2017; Walsh et al. 2015). Therefore, careful assessment of the entire separating membrane is important to make the correct diagnosis.

12.2.3 Assessment of the Placenta in Late Pregnancy

Early ultrasound examination, preferably in the first trimester, is important to reliably detect chorionicity, since in early pregnancy the amniotic and chorionic membranes have not yet fused. Patients who receive their first ultrasound examination before 14 weeks gestation are significantly less likely to be misdiagnosed. As gestational age increases, the risk of misdiagnosis increases by 10% per week (Blumenfeld et al. 2014). When faced with a single placental mass in an advanced same-sex twin pregnancy, it can be difficult to make a correct diagnosis of chorionicity. After the first trimester, the typical insertion signs of the separating membrane may be difficult to assess, and counting the individual layers may be difficult. In these cases, the only remaining option is to describe the thickness of the separating membrane, which, however, often does not lead to the correct diagnosis.

Diagnosis of an MCMA placenta is easier because in this case there is no separating membrane. In an MCMA placenta, the cord insertions are typically closer together than in MCDA placenta, and velamentous insertion is rare (Umur et al. 2003; Zhao et al. 2015a). The umbilical cords are usually tangled together.

Placenta praevia is also more common in DC twins compared to singletons and MC twins (OR = 1.5). This is attributed to the presence of two placental masses (Weis et al. 2012). In addition, the incidence of vasa praevia is higher in twins compared to singletons (Jauniaux et al. 2017). Screening for the presence of the aforementioned pathologies should be performed routinely.

12.2.4 Assessment of the Placenta after Delivery

Assessment of the placenta after delivery is the safest procedure to confirm chorionicity. When two separate placentas are present, it is a dichorial twin pregnancy. The dichorial separating membrane is thick and consists of three layers: two amniotic layers and an intervening chorionic layer. A monochorial separating membrane, on the other hand, is very thin and transparent and consists of only two amniotic membranes, which can be separated from each other and peeled off completely. A dichorial septum is thick and opaque, and although the two amniotic membranes can be separated, the middle chorionic layer is firmly attached to the placenta. If in doubt, histology of the separating membrane can assure chorionicity (Galjaard et al. 2014). As mentioned earlier, there is no separating membrane in monoamniotic twins, although there is often a small amniotic fold on the placental surface between the two closely spaced umbilical cord insertions (Fig. 12.4). Vascular anastomoses in monochorial placentas can be easily visualized postpartum by dye injection (Lewi et al. 2013). This simple technique has been described previously and can be performed by anyone interested.

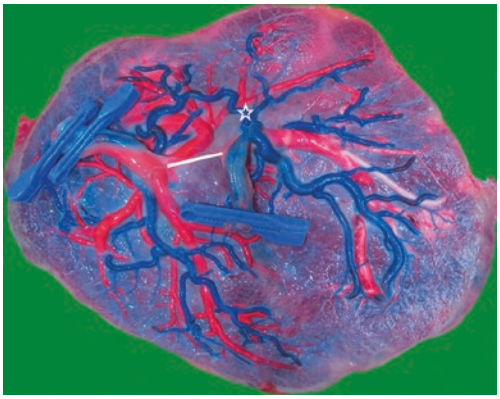

Fig. 12.4 MCMA twin placenta after dye injection. The arteries are stained blue, the veins of the first and second twin are white and red, respectively. The umbilical cords insert close to each other (*line*) and there is a large AA anastomosis (*asterisk*)

12.3 The Placenta in Twin-to-Twin Transfusion Syndrome (TTTS)

Twin-to-twin transfusion syndrome (TTTS) is an amniotic fluid discordance and involves donor oligohydramnios (deepest vertical amniotic fluid depot <2 cm) and acceptor polyuric polyhydramnios (>8 cm before 20 weeks of gestation and >10 cm thereafter) (Senat et al. 2004). A TTTS develops in approximately one in ten twin MCDA pregnancies usually between 16 and 26 weeks gestation (Couck et al. 2017a; Lewi et al. 2008a). TTTS is rare in MCMA pregnancies (Umur et al. 2003). The condition does not occur in dichorial twins because they do not have placental vascular anastomoses.

12.3.1 Vascular Anastomoses in TTTS

The underlying mechanism of TTTS is an imbalance of blood flow across the placental vascular anastomoses. The AV anastomoses

in TTTS allow unidirectional and thus potentially imbalanced blood flow. Almost all TTTS placentas have AV anastomoses. It is not yet known in detail how exactly these AV anastomoses cause TTTS, as neither the number nor the diameter of AV anastomoses is increased in TTTS placentas (De Paepe et al. 2010a).

Anastomoses between two arteries (AA) are thought to protect against TTTS. They are bidirectional and thus have the potential to correct imbalanced blood flow created by AV anastomoses. Because of their low resistance, they are more effective than AV anastomoses of the same size (Umur et al. 2002). AA anastomoses are less common in TTTS placentas compared to uncomplicated MC placentas (37% vs. 87–91%) (Lewi et al. 2007; de Villiers et al. 2012). While the presence of AA anastomoses lowers the risk of TTTS, spontaneous occlusion of an AA anastomosis can lead to acute TTTS (Tan et al. 2004). Stenoses of these AA anastomoses have also been associated with the development of TTTS (van den Wijngaard et al. 2008). Almost all MCMA placentas have an AA anastomosis and therefore they are less likely to develop TTTS (Umur et al. 2003).

Much less is known about the role of venovenous (VV) anastomoses in the development of TTTS. VV anastomoses appear to be slightly more common in TTTS placentas (36% vs. 25% in placentas without TTTS) (Lewi et al. 2007). Considering only placentas without AA anastomoses, 32% of TTTS placentas have VV anastomoses compared to only 8% of placentas without TTTS (Zhao et al. 2015b). VV anastomoses may therefore increase the risk of TTTS in the absence of AA anastomoses (de Villiers et al. 2015). One hypothesis is that veins are more susceptible to external pressure and that this may cause them to behave like a functional AV anastomosis rather than allowing bidirectional flow (Zhao et al. 2015b).

Placental anastomoses are causally responsible for TTTS. This is supported by the fact that closure of these anastomoses by laser coagulation terminates existing TTTS and is therefore the current therapy of choice (Senat et al. 2004). During laser coagulation, all anastomoses between donor and acceptor are closed. A sequential coagulation technique is sometimes used, in which AV anastomoses from the donor to the recipient are coagulated first, followed by those from the recipient to the donor. In this way, reverse transfusion from the hypervolemic recipient to the hypovolemic donor occurs during laser coagulation (Chmait et al. 2014). However, it is much more important to minimize the risk of missing anastomoses. This can be achieved by using the Solomon technique: After coagulating all visible anastomoses, the surgeon draws a coagulation line across the entire placenta and connects the coagulation areas from one placental margin to the other (Slaghekke et al. 2014). If large anastomoses are missed, this can again lead to TTTS or even intrauterine fetal death. Small missed anastomoses typically result in a twin anemia-polycythemia sequence (TAPS) after laser coagulation, characterized by a marked hemoglobin difference between fetuses (Lewi et al. 2006). The use of the Solomon technique reduces the risk of recurrent TTTS (1% vs. 7% with the standard technique) and TAPS (3% vs. 16%) (Slaghekke et al. 2014). Although it is undisputed that the anastomoses play a leading role in the development of TTTS, there must be more components to the disease than simply a shift in blood.

The affected twins usually do not show hemoglobin discordance (Saunders et al. 1991). Exchange of fluid from donor to acceptor through large placental anastomoses results in hypovolemia of the donor and hypervolemia of the recipient, which can mask anemia and polycythemia, respectively (Couck and Lewi 2016). Hormonal

factors are also likely to be involved in disease progression (Mahieu-Caputo et al. 2000; Bajoria et al. 2003).

12.3.2 Importance of the Umbilical Cord in TTTS

The significance of a velamentous umbilical cord insertion for the development of TTTS remains controversial. Almost all studies to date have been performed on placentas obtained postpartum. Most authors failed to demonstrate a link between a velamentous umbilical cord insertion and TTTS (de Villiers et al. 2012; Lopriore et al. 2007; Kent et al. 2011; Hack et al. 2008; Yonetani et al. 2015; Costa-Castro et al. 2013, 2016), while others reported a more frequent occurrence of TTTS with velamentous umbilical cord insertion (De Paepe et al. 2010a; Fries et al. 1993; Machin 1997). A fundamental problem in the interpretation of these studies is due to the retrospective design of postnatal placental series, which are prone to selection bias in favor of pregnancies with two surviving children.

A prenatal study assessing umbilical cord insertions by ultrasound at 12 and 16 weeks of gestation found evidence that a velamentous insertion increases the risk of TTTS in one or both twins (Couck et al. 2017a). It has been speculated that a velamentous insertion is more susceptible to compression, which could reduce blood flow and hereby lead to hemodynamic instability and TTTS (Fries et al. 1993). Although a velamentous cord insertion increases the risk of TTTS, this finding is not suitable for screening. This would detect only one in three pregnancies that go on to have TTTS (Couck et al. 2017a). If a TTTS develops in a velamentous umbilical cord insertion, this affects the donor's cord insertion in 80% of cases (Couck et al. 2017a).

12.3.3 Unequal Division of the Placenta in TTTS

Although unequal division of the placenta is not more common in TTTS, donors generally have a slightly smaller portion of the placenta than recipients (Lopriore et al. 2007). In cases where unequal division occurs, the donor almost always has the smaller placental portion (De Paepe et al. 2010a). Whether this is the cause or a consequence of TTTS remains to be seen. The decreased placental perfusion in the hypovolemic donor portion could lead to decreased expansion of placental cotyledons on the donor side (Lopriore et al. 2007).

12.3.4 Significance of Other Placental Factors for TTTS

An abnormal vascular distribution pattern is observed in 60% of TTTS placentas versus 44% in placentas without TTTS, with the donor more commonly affected than the recipient (87% vs. 33%) (De Paepe et al. 2005).

In magnetic resonance imaging (MRI), TTTS placentas show an abnormal maturation process. However, this has only been shown in one small study and also exists in two-thirds of placentas without TTTS (Linduska et al. 2012).

12.4 Monochorial Placenta and Discordant Growth

In contrast to TTTS, discordant growth or selective fetal growth restriction (sFGR) is not clearly defined. Most authors use a growth discordance of more than 20% or 25% relative to the larger fetus for this purpose (Ortibus et al. 2009; Van Mieghem et al. 2009; Chalouhi et al. 2013; Couck et al. 2017b). However, some define sFGR as growth of the smaller twin <10th percen-

tile for the respective gestational age (Parra-Cordero et al. 2015; Gratacos et al. 2008; Valsky et al. 2010). The International Society of Ultrasound in Obstetrics and Gynecology (ISUOG) recommends referral to a top-tier perinatal or prenatal center for intensified monitoring if the discrepancy between twins equals or exceeds 25% (Khalil et al. 2016). Even a discordance of 20% or more appears to increase perinatal mortality as well as the risk of preterm birth <32nd week of gestation. Growth discordance is calculated from estimated fetal weight (EFW) using the following formula: (EFW [larger twin] – EFW[smaller twin])/EFW[larger twin].

The incidence of sFGR varies from 8% with a cut-off of 25%, to 15% with a cut-off of 20%, depending on the definition used (Couck et al. 2017b). The main diagnosis of discordant growth always implies the absence of TTTS. Although the smaller twin usually has less amniotic fluid than the larger twin, the difference is not so marked as to meet the criteria for TTTS.

Depending on whether diagnosed before or after the 26th week of gestation, early growth discordance is distinguished from late growth discordance. In addition, sFGR can be classified according to the Doppler parameters in the umbilical arteries (UA) of the smaller twin.

- Type I includes sFGR cases with positive end-diastolic flow (EDF) in the UA (Fig. 12.5),
- Type II includes sFGR cases with persistent absent or reversed EDF (AREDF) (Fig. 12.6), and
- Cases with intermittent AREDF are classified as type III (Fig. 12.7).

These sFGR types not only differ in terms of pregnancy outcome, but also show different placental characteristics (Gratacos et al. 2007). Pathological Doppler parameters occur frequently in early onset sFGR, while they are rare in the late form (Lewi et al. 2008b).

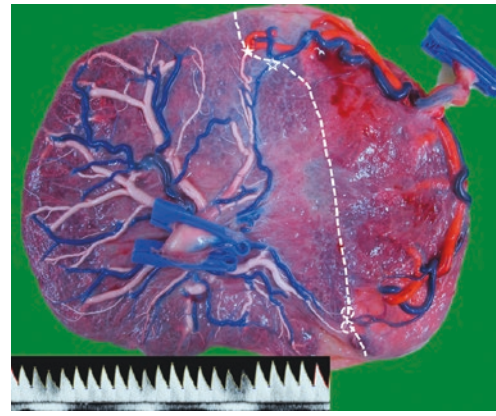

Fig. 12.5 sFGR type I. In this pregnancy, discordant growth in favor of the twin with the central umbilical cord attachment was evident from the 21st week of gestation. Umbilical artery Doppler parameters (*bottom*) in the smaller twin were normal during pregnancy. In the 34th week of gestation labor started spontaneously; two healthy girls were born with 2255 g and 1780 g respectively (21% discordance). Twin 1 (*left*) has a central umbilical cord insertion, while twin 2 (*right*) has a marginal umbilical cord insertion. The placenta is unevenly distributed. The arteries of both twins were injected with blue dye, and one AA anastomosis is present (*empty asterisk*). The veins of twin 1 and 2 are white and pink. The *dashed line* indicates the vascular equator (separation of the two placental portions). One VV-anastomosis (*filled asterisk*) and two VA-anastomoses (*empty circles*) are present

12.4.1 Unequal Division of the Placenta and Discordant Growth

The degree of unequal distribution is greater in placentas of twins with discordant birth weight than in those with concordant birth weight (De Paepe et al. 2010b). Furthermore, placental partitioning differs between early and late onset growth discordance. Although both may eventually lead to a similar discrepancy in birth weight, late sFGR placen-

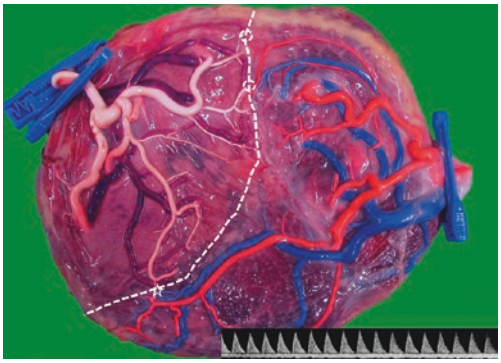

Fig. 12.6 sFGR type II. Placenta of a pregnancy with severe growth discordance from the 17th week. While the UA Doppler of the smaller twin was normal at first, it deteriorated to continuous end-diastolic absent flow at 27 weeks of gestation. Due to growth arrest, cesarean section was performed at 30 + 5 weeks of gestation. Two boys were born at 1588 g (*one clamp*) and 805 g (*two clamps*) (49% discordance). The placenta is moderately discordant. Arteries are stained pink and white, veins blue and purple for twin 1 and 2, respectively. There is a small AA anastomosis. *Asterisk* and *circles* indicate the present anastomoses, the *dashed line* the vascular equator

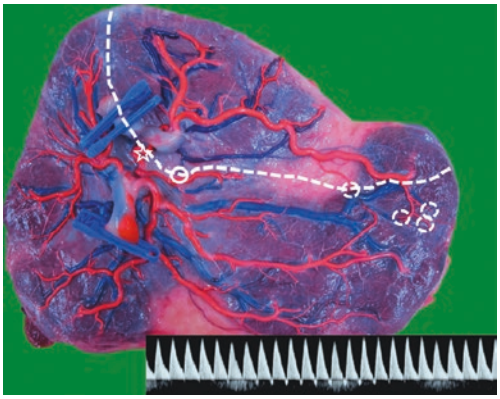

Fig. 12.7 sFGR type III. At 16 weeks of gestation, growth was diagnosed with 24% discordance. From 23 weeks onwards, there was intermittent absent or reversed flow in the UA of the smaller twin. Concurrently, there was polyhydramnios in the larger twin (deepest vertical deposit of 9 cm) with reduced but normal amniotic fluid in the other twin. Due to a concomitant shortening of the cervix to only 9 mm, an amniotic drainage was performed in the larger twin (850 ml). In the 30th week of gestation there was spontaneous onset of labor; 2 boys were born with 1585 g and 1300 g (18% discordance). The placenta is very unevenly distributed. The arteries of both twins are stained red, the veins of twin 1 (*one clamp*) are blue and those of twin 2 (*two clamps*) are purple. There is a large AA anastomosis. The *dashed line* indicates the vascular equator (separation of the two parts of the placenta), the *asterisk* and *circles* indicate the present anastomoses

tas only show minor differences in their partitioning compared to those with concordant growth. In contrast, early sFGR placentas are typically unequally partitioned (Lewi et al. 2008b). This unequal partitioning intensifies with sFGR type. While in concordant growth the ratio of the two placental areas differs by a factor of 1.3, this factor increases to 1.8 in sFGR type I, 2.6 in type II, and 4.4 in type III. Although the discrepancy in placental areas is more pronounced in sFGR type III compared to type II, the fetal weight discordance behaves similarly in both groups (Gratacos et al. 2007).

12.4.2 Anastomoses and Discordant Growth

Compared to placentas with early sFGR, placentas with late sFGR typically have smaller AA anastomoses and a smaller overall diameter of all anastomoses. Presumably, therefore, late sFGR is associated with twin anemia-polycythemia sequence (TAPS) in 38% of cases, whereas it is rare in early sFGR (Lewi et al. 2008b).

While the proportion of placentas with AA anastomoses in type I and type II sFGR does not differ from that in normal MC placentas, AA anastomoses are present in all sFGR type III placentas and are responsible for the classic intermittent Doppler pattern. These AA anastomoses are significantly larger in sFGR type III placentas than in normal placentas. In contrast, sFGR type II placentas are more characterized by small AA anastomoses. This explains the differences in pregnancy outcome. While both

show highly discordant growth, the large AA anastomoses in type III placentas allow massive and sudden volume shifts. These may lead to unpredictable intrauterine death or brain damage of a fetus, while the small AA anastomoses in type II placentas allow only slow transfusion. Therefore, outcome in type II cases is more predictable, and progressive deterioration of Doppler parameters in the growth-retarded twin can be expected, similar to that in a growth-retarded singleton (Gratacos et al. 2007).

In contrast, the number of AV and VV anastomoses does not differ between normal placentas and placentas in early sFGR, regardless of the type of UA Doppler (Gratacos et al. 2007).

12.4.3 Umbilical Cord and Growth Discordance

Velamentous umbilical cord insertion in MC placentas has been described with discordant growth in both postnatal and prenatal studies (Couck et al. 2017a; Kent et al. 2011; Costa-Castro et al. 2013, 2016; De Paepe et al. 2010b; Hanley et al. 2002). Both a velamentous cord insertion and discordance between the two cord insertions are independent predictors of discordant growth. The risk for discordant growth is higher when a velamentous cord insertion in one twin is combined with a central cord insertion in the other. In cases with discordant growth and cord insertions, the central attachment belongs to the larger twin in 92% of cases. Unfortunately, both criteria are not suitable as screening methods for discordant growth (Couck et al. 2017a).

A singular umbilical artery (SUA) is associated with reduced fetal growth. This results in a marked discordance in birth weights in discordant twins (Stout et al. 2013; Klatt et al. 2012). A large prenatal ultrasound study showed that SUA is present in approximately 2% of MC and DC twins (Stout et al. 2013).

12.4.4 Molecular Changes and Discordant Growth

In MC twins, oxidative stress may play a role in the development of sFGR. Markers of oxidative stress are elevated in the placental area of the sFGR twin (Wu et al. 2017; Zhang et al. 2015). However, it is unclear exactly how this mechanism works. For example, nuclear factor erythroid 2 like 2 (NFE2L2) is upregulated in the smaller twin placenta in sFGR type I, whereas it is downregulated in singletons with fetal growth restriction. Since NFE2L2 indicates mild hypoxia, it may be that hypoxia in the smaller twin's placenta is alleviated by transfusion from the larger twin via the vascular anastomoses. This is not possible in singletons, which is why their placentas are more likely to be affected by severe hypoxia (Wu et al. 2017). Several other factors, including leptin, appear to be upregulated in the placental areas of smaller twins (Sun et al. 2017).

Although the molecular analysis of the sFGR placenta is a novel and exciting field of research, previous studies have only been able to demonstrate differences between the different placental areas. However, the exact mechanism by which markers of oxidative stress lead to sFGR is still unknown.

12.4.5 Other Placental Factors and Discordant Growth

Arteries and veins of the smaller sFGR-MC twins have a reduced branching pattern compared to larger and normal MC twins (Gou et al. 2017).

Although pathological placental lesions (such as infarcts and chorangiomas) are more common in MC twins than in DC twins, they are not clustered in sFGR-MC twins (Kent et al. 2012). Only fetal vascular thrombosis was observed more frequently in MC-sFGR twins in a small study (Chan et al. 2010).

12.5 Dichorial Placenta and Discordant Growth

While MC twins are monozygotic and thus have the same genetic growth potential, DC twins are mostly dizygotic. Therefore, differences in their growth may be partly related to different genetic predispositions. The DC placenta does not have placental anastomoses, so the fate of the twins is not directly linked. Nevertheless, the ISUOG also suggests intensified monitoring here starting from a weight difference of 25% (Khalil et al. 2016).

12.5.1 Umbilical Cord and Discordant Growth

The incidence of velamentous cord insertions in DC placentas is much lower than in MC placentas (8% vs. 37–40%). In contrast to MC twins, velamentous umbilical cord insertion in DC placentas does not appear to be associated with lower birth weight or severe birth weight discordance, but is associated with lower gestational age at birth (Costa-Castro et al. 2016).

As in MC twins, a singular umbilical artery (SUA) is associated with a fetus that is too small for gestational age (SGA). The incidence of SUA is similar in DC and MC twins (Stout et al. 2013; Klatt et al. 2012).

12.5.2 Placental Pathology and Discordant Growth

Pathologic placental lesions (defined as at least one of the following: placental infarction, chorangioma, subchorial fibrin deposition, retroplacental hematoma, and/or impaired placental maturation) are also more common in the smaller twin of a discordant dichorial twin than in the placenta of the larger twin or in normal twin pregnancies. In addition, pathological placental lesions are almost twice as common in twins with birth weight < fifth percentile compared with those with birth weight equivalent to gestational age (58% vs. 33%) (Kent et al. 2011). In contrast to MC twins, fetal vascular thrombosis in DC twins is not associated with FGR but is associated with hypertensive disease (Chan et al. 2010). The level of apoptosis is increased in the placenta of the smaller twin compared to the larger twin, suggesting a role of oxidative stress in discordant growth in DC twins (Almog et al. 2002).

One study reported a small but significant difference in birth weight between the firstborn and secondborn twin in DC twins in favor of the leading twin. In this series, the obstetric trailing twins were more likely to have a small placenta (31% vs. 9%) and twice as likely to have maternal perfusion defects such as decidual vasculopathy, villous infarcts, premature villous maturation, or increased intervillous fibrin deposits (42% vs. 21%) (Weiner et al. 2017).

12.6 Conclusion

Monochorial and dichorial placentas differ significantly in both architecture and function. In both, placental abnormalities can lead to different pathologies. The early accurate diagnosis of chorionicity is essential for the correct assessment of risk constellations and for the competent management of a twin pregnancy.

- Determination of chorionicity and amnionicity in the first trimester is essential for the management of twin pregnancies. This can be easily done by assessing the separating membrane between the twins.
- Examination of the placenta after birth remains the gold standard to confirm chorionicity. Dye injection can be used to visualize the vascular anastomoses in monochorial placentas.

- The most important difference between monochorial and dichorial placentas is the existence of vascular anastomoses in the monochorial placenta.
- Twin-to-twin transfusion syndrome (TTTS) is defined by marked discordance of amniotic fluid volumes caused by imbalanced blood flow across vascular anastomoses in monochorial twins.
- The mechanism of discordant growth in multiples is different in monochorial and dichorial twins.
- In monochorial twins, unequal division of the placenta, vascular anastomoses, and velamentous cord insertion are associated with discordant growth.
- Placental lesions are more frequently present in discordant growth of dichorial twins.
- A singular umbilical artery is associated with growth discordance in both monochorial and dichorial twins.

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Fetal Programming

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Contents

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13.1 Introduction

For a long time it was assumed that the risk for diseases such as coronary heart disease (CHD) or insulin resistance, for example, develops from the genetic potential of the parents and is amplified by environmental influences such as an unfavorable lifestyle. This view has been completely overturned in the last two decades by the concept of fetal programming. The concept implies that a stimulus or insult during a sensitive period of fetal development produces permanent changes in structure, physiology, and metabolism, determining later risk for chronic diseases such as CHD and insulin resistance, as well as allergies, an impaired stress response, and many others in adulthood. The concept of Fetal Origins of Adult Disease (FOAD) was introduced by the British epidemiologist David Barker more than 20 years ago and has been the subject of intensive research ever since. The current research approaches aim to identify the biological mechanisms by which a prenatal stimulus or insult alters fetal development, the time lag between prenatal stimulus/insult and later disease, and the numerous factors that contribute to disease risk throughout the lifespan. From the initial observations, a separate branch of research, the Developmental Origins of Health and Disease (DOHaD), has now developed with its own international society.

The FOAD is based on the concept of developmental plasticity. According to this, an organism is sensitive to influences in an early and critical developmental phase in order to provide the best adaptation of the phenotype to the expected environment. For example, an insult such as maternal malnutrition during pregnancy results in altered organ morphologies and functions with the goal of ensuring general fetal as well as neurological development and survival. These changes are intended to prepare the fetus for life outside the mother and for possible stressors after birth (► Sect. 13.2.1). This developmental plasticity is likely to be largely lost by preschool/school age.

The aim of this chapter is to provide an overview of the most important epidemiological and experimental studies and to elaborate the possible underlying pathophysiological mechanisms of the FOAD hypothesis. In this context, the placenta, as a key regulatory and barrier organ between father, mother and fetus, plays a crucial role in the development of later chronic diseases. The most important findings on adaptively altered placental morphology and function and disturbed fetal development are highlighted and discussed in the individual sections.

13.2 Between Hypothesis and Epidemiology

13.2.1 This Is How It All Began: The Barker Hypothesis and First Epidemiological Studies

From early epidemiological studies linking high infant mortality with later ischemic heart disease in survivors (Forsdahl 1977), it was postulated that negative influences may be present during pregnancy and early childhood that promote the later development of cardiovascular disease. Barker developed the FOAD hypothesis in 1995 based on his analysis of large data collections of the birth cohorts of the 1920s and 1930s in England and Wales, which showed that low birth weight – as an expression of poor fetal development/maturation – is associated with hypertension, impaired glucose tolerance, and increased mortality from cardiovascular disease and cerebral ischemic insults later in life (Barker 2004). Birth weight is not per se the cause of increased disease risk; rather, low birth weight reflects developmental conditions in the womb that influence the physiology of the growing

organism and subsequent disease risk. In a large study of 16,000 women and men, all-cause mortality was found to be independent of weight and height at birth, but cardiovascular disease mortality was halved from lowest to highest birth weight (Osmond et al. 1993). A small increase in mortality at the highest weight percentiles was associated with macrosomia rates in gestational diabetes (U-shaped progression of mortality). Subsequently, large cohort studies, such as the large-scale American Nurses Health Study (Curhan et al. 1996), confirmed that the risk of CHD and ischemic cerebral insult is associated with low birth weight (relative risk increases by 0.85/kg of birth weight). Corresponding to body size at birth, an association between placental size and shape and the risk of CHD or sudden cardiac death has also been demonstrated (Barker et al. 2012). Thus, small placentas as well as large placental weight/birth weight ratios (as a sign of placental performance) were particularly correlated with cardiovascular disease and hypertension (Barker et al. 1990). A good overview of the association of placental morphology and later disease development is provided by two recent reviews (Thornburg et al. 2016; Burton et al. 2016).

Barker further postulated that an insufficient supply of nutrients to the fetus and/or infant was mainly responsible for the predisposition to later chronic diseases. Hypertension, dyslipidemia, and other risk factors for cardiovascular disease such as impaired glucose tolerance or type 2 diabetes mellitus are often grouped together as syndrome X or metabolic syndrome and show an association with small body size and low birth weight. Barker therefore gave this constellation of diseases the name "the small baby syndrome" (Barker et al. 1993).

Low birth weight is often due to fetal growth restriction (FGR), which may be due to maternal nutritional deficiency, placental insufficiency, fetal abnormalities, maternal stress, or external toxins such as nicotine abuse or drugs. In this context, low birth weight is an example – albeit an important one – of negative influences during pregnancy and thus an "early" indicator of possible later disease development. However, even with normal or increased birth weight, FOAD of organ systems such as the immunological, metabolic or humoral system can take place. An overview of important prenatal stimuli/insults and the discussed associations with later disease is given in ◘ Fig. 13.1. In most epidemiological studies, only one trigger at a time is investigated in relation to a later disease or group of diseases. Mutual influences and/or complementary effects of the different stimuli/insults are very likely, but still largely unexplored.

Often, not only the stimuli alone, but also the timing, duration of exposure and intensity, as well as the developmental stage of the various tissues and organs are important influencing factors of FOAD. The kidneys, for example, will be most susceptible to external stimuli/insults during the phase of nephrogenesis, whereas the brain grows and differentiates during the prenatal period and also during infancy and is thus vulnerable for a much longer time. Stimuli/insults cause intrauterine tissue restructuring. This often results in a decrease in organ mass and total cell number (e.g. thymic hypoplasia, reduced number of nephrons of the kidney and β-cells in the pancreas, etc.).

> The consequences of intrauterine programming are often manifested by a decrease in organ mass and/or total cell count.

Using two post-war cohorts that at first glance appear very similar, we will demonstrate the possible different effects of the same stimulus:

- During the relatively short Dutch famine winters of the war years 1944/45, daily food intake was limited to 400–1000 calories. The timing of maternal famine in relation to gestational age (first, second,

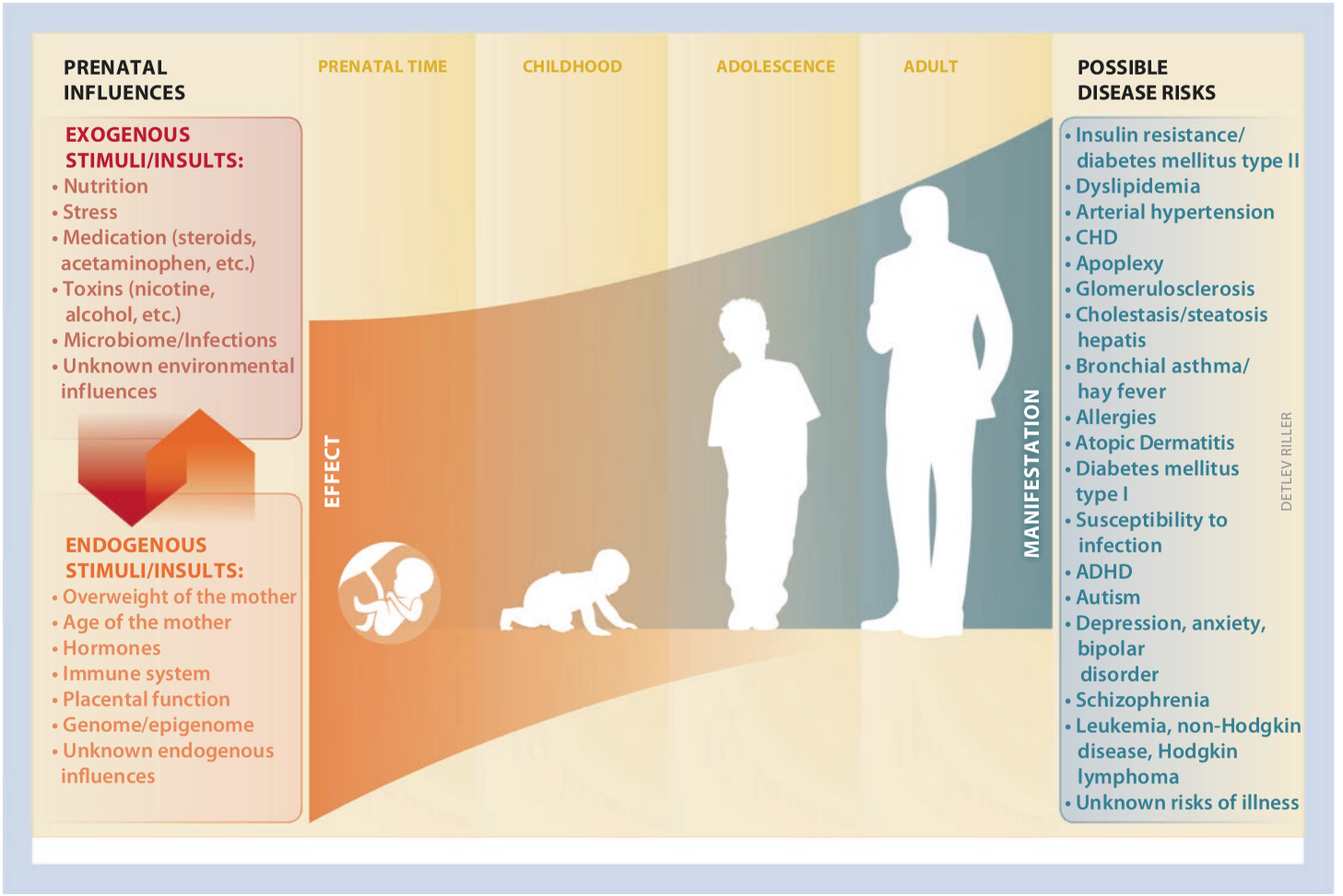


Fig. 13.1 Overview of prenatal influences and their association with later disease development. *ADHD* attention deficit hyperactivity disorder; *CHD* coronary heart disease

third trimester of pregnancy) had different effects on the development of chronic diseases. Newborns who were exposed to malnutrition in the last months before birth were lighter at birth and showed reduced glucose tolerance later in life. Infants whose mothers were starved in early pregnancy but subsequently had normalized caloric intake during the remainder of pregnancy had normal birth weights but atherogenic lipid profiles and higher BMI later in life (Ravelli et al. 1976; Roseboom et al. 2000).

- The Leningrad Blockade of 1941–1944 and the associated famine lasted a total of 800 days. Children at that time were exposed to hunger not only throughout pregnancy but also in the first years of life. Contrary to the observations in the Dutch study, however, girls and boys did not develop hypertension, CHD, dyslipidemia and insulin resistance in adulthood (Stanner et al. 1997).

Although the two cohorts appear similar, the famines differed in severity and duration. Newborns who survived the maternal shortage in Leningrad developed an "energy-saving mode" ("thrifty phenotype") that helped them survive the postnatal shortage (Hales and Barker 2001). These children were well prepared for the future crisis situation already intrauterine, and their adaptations ("predictive adaptive responses") might have acted as protection against chronic diseases. In contrast, the Dutch children showed accelerated weight gain in the first years of life. The intrauterine environment had prepared the children for a significantly worse supply situation, which was not present after delivery.

> The same stimulus/insult can have different effects on the growing organism at different times in pregnancy.

This maladaptation during pregnancy was seen by Gluckman and Hanson as a trigger for the predisposition to later chronic diseases within the framework of the mismatch concept (Godfrey et al. 2007). Thus, one genotype can result in different expressions of the same phenotype due to early environmental influences.

13.2.2 Obesity, Insulin Resistance and Metabolic Syndrome

The mismatch concept can be applied not only to extreme situations such as famines, but can also be applied to our way of life today. Accelerated weight or size growth is the normal biological response of a developing organism to a period of malnutrition. Infants born small show 90% increased weight gain ("catch-up growth") after birth. However, the mismatch theory is potentiated by increasingly unhealthy diets and physical inactivity – problems that increasingly occur even in infancy. Human and animal evidence shows that for neonates with low birth weight and increased catch-up weight in childhood, the risk for metabolic syndrome or a disease component(s) such as hypertension, obesity, and/or insulin resistance/diabetes mellitus type 2 is increased. For both obesity and impaired insulin resistance, this predisposition can be shown epidemiologically and in animal experiments even beyond the next generation (Painter et al. 2008; Jimenez-Chillaron et al. 2009; Thamotharan et al. 2007).

> The predisposition of certain chronic diseases can be inherited beyond the next generation.

Low birth weight children who subsequently develop type 2 diabetes mellitus show increased hepatic glucose neogenesis due to increased phosphoenolpyruvate carbokinase (PEPCK) activity (Peterside et al. 2003), as well as decreased number and function of pancreatic β-cells (due to decreased proliferation and increased apoptosis) with reduced

insulin production and a high glucose/insulin ratio, which in turn result in insulin resistance of end organs (skeletal muscle, liver, and adipocytes) (Jensen et al. 2008; Ozanne et al. 2003). These changes can be demonstrated in animal experiments even in utero (Garofano et al. 1997; Schwitzgebel et al. 2009). The children tend to store fat on the trunk (visceral or trunk obesity) (Okosun et al. 2000; Ozanne et al. 2000). Adipocytes show decreased insulin-dependent glucose uptake as well as decreased metabolism of free fatty acids (Jaquet et al. 2000; Ozanne et al. 1997, 2001). Fatty deposits in the liver can then turn into permanent structural liver changes (hepatic steatosis) (Nobili et al. 2007; Muramatsu-Kato et al. 2015).

Not only the effect of insulin, but also that of leptin is altered. The hormone leptin is secreted in the placenta and adipocytes and is responsible for inhibiting food intake (appetite control in the hippocampus via neuropeptide Y) and increased energy expenditure via the central nervous system. Low birth weight infants have lower leptin concentrations at birth, but as catch-up weight increases, blood leptin concentrations increase, acting on progressively lower and later resistant hypothalamic leptin receptors. A condition results that is thought to be responsible for hyperphagia and decreased energy expenditure. Dysregulated insulin and leptin signaling pathways are two important examples of how low birth weight can result in type 2 diabetes mellitus and obesity.

13.2.3 Cardiovascular Diseases

According to Barker, the risk of cardiovascular disease is increased when low birth weight males catch up to weight in adolescence (in females from infancy). Height also remains below the overall average in adulthood, an aspect known to be associated with CHD. Low birth weight is also associated with increased systolic blood pressure in adolescents (Nilsson et al. 1997). This correlation persists even when adjusted for socioeconomic status (Bergvall et al. 2005). Similar to observations in the later development of insulin resistance, both CHD and hypertension are not associated with absolute BMI levels in childhood, but from the combination of BMI at birth and in childhood/adulthood.

Children with reduced birth weight show impaired cardiac and vascular function: they are more likely to show concentric left ventricular hypertrophy, a phenomenon also associated with CHD (Vijayakumar et al. 1995) and endothelial dysfunction (Leeson et al. 2001). The authors postulate that in terms of the risk of impaired endothelial function as a precursor to atherosclerosis, 1 kg difference in birth weight is equivalent to 4.5 years of smoking. Even in utero, this devastating remodeling of the vascular walls appears to begin, as demonstrated by the intima-media thickness of the fetal aortic wall in growth-restricted children (Zanardo et al. 2013; Gomez-Roig et al. 2015). Endothelial dysfunction is due to increased vasoconstrictive effects in animal models of food restriction, placental insufficiency, and hypoxia. Decreased expression of endothelial nitric oxide synthase (NOS) or soluble guanylate cyclase appears to reduce the availability of the vasodilator NO (Payne et al. 2004; Williams et al. 2005). Thus, increased peripheral vascular pressure and, in the long term, the development of hypertension are preprogrammed due to a limited relaxation capacity of the vascular walls. It should be noted that endothelial NOS can also be downregulated via the action of glucocorticoids (Wallerath et al. 1999).

Suboptimal renal development leading to reduced renal volume and nephron number is a known pattern in the later development of hypertension and cardiovascular disease (Singh and Denton 2015), an observation that is not consistent across studies but has also been confirmed in autopsies of primary hypertensives (Keller et al. 2003). Reduction in nephron number can be induced by food/pro-

tein restriction, glucocorticoid excess, hypoxia, or iron deficiency. Growth retarded animal fetuses show reduced but hypertrophic glomeruli and develop chronic kidney disease with hypertension later in life (Singh and Denton 2015). In contrast, it has been demonstrated that in transforming growth factor (TGF)-β2-heterogenic mice (an important growth factor in nephrogenesis), a high number of nephrons protects against later hypertension. At birth, nephrons must be able to take over the task of hemostasis of extracellular fluid from the placenta. Over the first 2 years of life, glomerular filtration rate (GFR) increases 25-fold. GFR is increased by approximately 10% via increased capillary pressure, 5% via increased permeability of glomerular capillaries, but mainly via expansion of glomerular filtration area (Singh and Denton 2015). According to the widely accepted Brenner hypothesis, reduced nephron number results pathophysiologically in decreased glomerular filtration area with sodium retention and secondary hypervolemia, which consecutively end in systemic hypertension and glomerulosclerosis (with again decreased glomerular filtration area) via increased glomerular capillary pressure – and thus a vicious circle. In addition to the reduced nephron number, however, dysregulation of the systemic and intrarenal renin-angiotensin system, increased sympathetic-neurological activity of the kidney, and increased tubular salt absorption appear to be possible factors in the development of subsequent renal disease and hypertension. These pathophysiological adaptations underscore the complexity of the signaling pathways involved that may be responsible for the fetal programming of cardiovascular disease and hypertension.

13.2.4 Altered Immune Response and Autoimmune Diseases

During pregnancy, the maternal immune system develops an active immune tolerance to fetoplacental antigens to accept the semi-allogenic fetus and protect it from rejection. This immune tolerance is supported by pregnancy hormones, such as progesterone and cortisol.

The child's immune system also develops during the fetal period. This includes the development of the acquired immune system, which ensures that the body's own structures can be distinguished from foreign antigens and that immunity – albeit not yet fully developed – to pathogenic germs can be built up at the time of birth (Cupedo et al. 2005). The fetal immune system in the first and second trimesters is rich in a specific T cell subpopulation, the anti-inflammatory regulatory T cells (Treg). Treg decline towards the end of pregnancy and reach low adult levels at birth (Wing and Sakaguchi 2010). Recently, it has been shown that Treg can maintain their functionality into adulthood (Palmer and Naeher 2009). The development of chronic immunological diseases such as allergies appears to be associated with reduced numbers of Treg, which in turn produce fewer T helper (Th)-1 cytokines in favor of Th-2 cytokine weighting (Solano et al. 2011). Th-2 cytokines are suspected of triggering allergization.

The aim of numerous research projects is currently to identify prenatal factors that influence the maturation of the fetal immune system and alter the subsequent risk of immunological diseases in children. In this context, the bacterial milieu of the maternal environment during pregnancy has been identified as a formative factor, as children born in a rural environment have a lower risk of allergies than children from big cities (von Mutius and Vercelli 2010). Already in the umbilical cord blood of children born in rural areas, both an improved immunological function and an increased number of Treg can be detected, probably as an indication of a prenatal sensitization of the immune system by an increased number of stimuli or allergens.

In contrast, prenatal stress exposure is suspected to increase the risk of asthma and allergies in the later life of the offspring. On the one hand, stress appears to alter the profile of inflammatory cytokines and the Th-1/Th-2 balance in the longer term (Solano et al. 2011). On the other hand, elevated endogenous glucocorticoid levels triggered by increased stress levels in the expectant mother are also seen as a significant trigger for the steady increase in atopic and allergic diseases in childhood (Solano et al. 2011). Responsible seems to be not only an increased glucocorticoid level induced by stress (Sect. "hypothalamic-pituitary-adrenal axis"), but also a decreased maternal progesterone level leading to an altered phenotype of maternal immune cells and an exaggerated inflammatory reaction between fetal and maternal circulation of the placenta. It has been shown that lowered progesterone levels in the first trimester are associated with increased susceptibility to atopic or allergic disease (Pincus et al. 2010; Hartwig et al. 2014). Maternal progesterone levels may also be decreased by nicotine abuse. Tobacco use during pregnancy is a best known risk factor for the development of hay fever and asthma (Gibbs et al. 2016). Meanwhile, epigenetic changes in the offspring of female smokers are also found to promote the development of asthma (Scholtens et al. 2014). Another external stimulus that has been linked to the development of hay fever is the use of acetaminophen during pregnancy (Eyers et al. 2011). The causal relationships are as yet insufficiently explored.

13.2.5 Memory and Psychiatric Disorders

Not only is the child's immune system susceptible to maternal stress, but the brain development of the fetus can also be significantly influenced by early experiences of stress. Stress and associated disorders such as maternal depression and anxiety during pregnancy appear to be risk factors for the later development of autism, anxiety and attention disorders, addictiveness, depression, schizophrenia, and impaired cognitive development in the offspring (Glover 2011). The development and maturation of the brain as well as most other organs are dependent on maternal glucocorticoids. Glucocorticoids affect neuronal migration, synapse formation, and neurotransmitter activity (Weinstock 2008). Excessive glucocorticoids, such as in chronic maternal stress, can be detrimental not only via reprogramming of the hypothalamic-pituitary-adrenal (HPA) axis but also via altered brain structure and function. In a prospective study of 125 pregnancies, amniotic fluid cortisol levels were inversely correlated with cognitive development at 17 months of age (Bergman et al. 2010). Furthermore, in animal experiments, the offspring of stressed female rats showed a tendency towards anxiety and depressive behavior (Welberg and Seckl 2000).

The amygdala and hippocampus are important components of the limbic system and thus primarily responsible for stress processing and response, cognition (both learning and remembering), and the processing of emotions such as fear and threat. For example, increased levels of the stress hormone cortisol in the saliva of pregnant women were shown to be associated with increased amygdala volume and affective disorders in girls at the age of seven (Buss et al. 2012). Reduced volumes of the hippocampi have been demonstrated primarily after prenatal maternal anxiety (Qiu et al. 2013). The hippocampus is particularly rich in glucocorticoid receptors (GR) and mineralocorticoid receptors (MR). These GRs and MRs are thought to be targets for epigenetic processes. For example, animal experiments in guinea pigs demonstrated increased expression of GR and MR

mRNA in the hippocampus (Dunn et al. 2010). Other morphological changes included a thinned cortex of the frontal lobe, which was seen primarily in offspring whose mothers reported depressive symptoms during pregnancy (Sandman et al. 2015). Changes in the connections of the limbic system (stress and anxiety management) (Rifkin-Graboi et al. 2013) or the corticolimbic system (Qiu et al. 2015) (here, late preterm infants >35 weeks of gestation were also included) have also recently been demonstrated using functional magnetic resonance imaging.

13.2.6 Gender-specific Programming

Many prenatal influences such as maternal malnutrition or stress etc. seem to affect male and female fetuses differently. The reasons for this are often not widely understood, but differences in placental function, adrenocortical hormones and testosterone, various epigenetic changes, sex-dependent responses of the transcriptome or sexually dimorphic development such as during brain development seem to play a role here (Werling et al. 2016; Frahm et al. 2016). Sex-specific differences in genetic expression also appear to underlie differential placental responses to external stimuli. For example, girls whose mothers suffered from anxiety and depression were found to have lower levels of mRNA encoding the enzyme 11β-hydroxysteroid dehydrogenase 2 (11β-HSD2), an enzyme important for glucocorticoid metabolism into the more inactive form of cortisone (Mina et al. 2015). This can be seen as a possible reason why girls are exposed to elevated glucocorticoid levels in these situations. Whether the reduced expression of 11β-HSD2 is related to the subsequent elevated cortisol levels for the affective disorders commonly observed in girls (Buss et al. 2012) remains to be investigated.

In contrast, maternal stress, especially in the first trimester, increases the risk of later schizophrenia, especially in the male offspring (Khashan et al. 2008). This risk seems to be increased by hypoxia, infection and maternal malnutrition (Buka et al. 2001; Cannon et al. 2002; Susser et al. 2008). Prenatal stress especially in the period between 21–32 weeks of gestation and depression have also been associated with the later development of autism in boys.

13.3 Underlying Mechanisms

The pathophysiological mechanisms underlying early development and disease risks later in life are complex, multidimensional, and incompletely understood. Some important hypotheses have been proposed. It is likely that many adaptations act in a complementary manner that are responsible for later disease development. Here, the currently most important theories are presented as examples:

13.3.1 Direct Mother-to-Child Mediators

Maternal Hormones, Growth Factors and Cytokines

Hypothalamic-Pituitary-Adrenal (HPA) Axis

Adrenal glucocorticoids are responsible for the development and maturation of fetal organs and increase continuously throughout pregnancy, with an exponential increase towards birth (Duthie and Reynolds 2013). From the second trimester onwards, the circadian secretion of pituitary adrenocorticotropic hormone (ACTH) is not only stimulated by the hypothalamic hormones corticotropin releasing hormone (CRH) and vasopressin (AVP), but the placenta also secretes CRH (◘ Fig. 13.2). Placental CRH

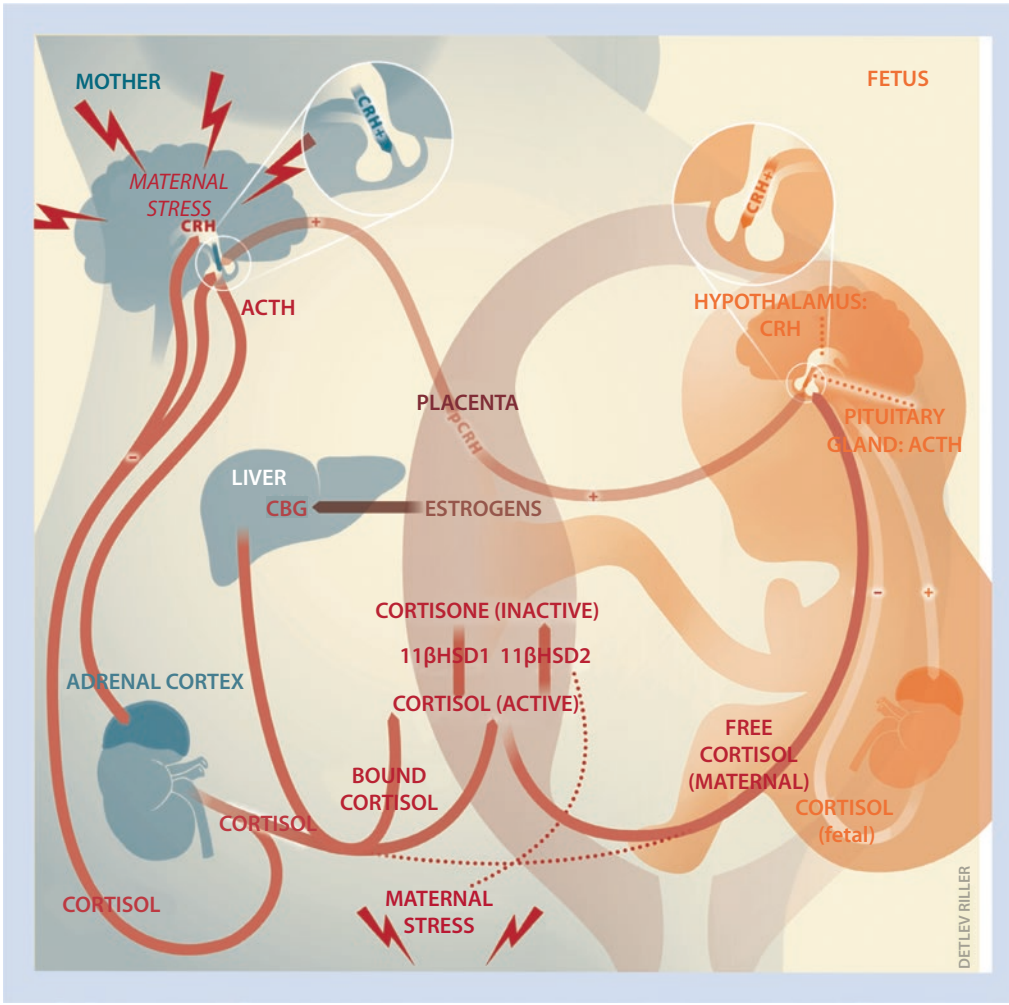


Fig. 13.2 Maternal and fetal hypothalamic-pituitary-adrenocortical axis. The fetal and maternal hypothalamic-pituitary-adrenal (HPA) axes communicate in an endocrine way across the placenta. The pCRH stimulates both maternal and fetal cortisol production. Both maternal and fetal cortisols in turn influence pCRH in a positive feedback mechanism (not shown). Placental 11β-HSD2 inactivates maternal cortisol by oxidizing it to cortisone (which has a lower affinity for glucocorticoid and mineralocorticoid receptors), thus protecting the fetus from maternal cortisol excess. The fetus develops the ability to produce its own hormones in late pregnancy. Both fetal ACTH and cortisol concentrations increase until birth, triggering the actual birth process. *11β-HSD1 and 2* 11β-hydroxysteroid dehydrogenase types 1 and 2, *ACTH* adrenocorticotropic hormone, *CBG* cortisol-binding globulin, *CRH* corticotropin releasing hormone, *pCRH* placental corticotropin releasing hormone

can act directly on the myometrium and is a potent vasodilator. However, in contrast to hypothalamic CRH, the secretion of placental CRH is not inhibited but stimulated by maternal and fetal cortisol (positive feed-forward mechanism). Cortisol, in turn, sometimes causes not only a negative feedback mechanism on CRH in the amygdala, but also stimulates central norepinephrine production and activation of the peripheral sympathetic nervous system. The placenta additionally synthesizes estrogen and pro-

gesterone, both of which influence cortisol levels. Estrogen stimulates hepatic production of cortisol-binding globulin (CBG), to which cortisol is bound in the circulation. Progesterone displaces cortisol from binding CBG and curbs placental CRH production. Glucocorticoids exert their effects peripherally in fetal tissues via intracellular GR or with higher affinity via restricted expressed MR. Glucocorticoids are lipophilic and can cross the placental barrier unimpeded. However, fetal glucocorticoid levels are only one-fifth to one-tenth of maternal levels and are controlled by expression of the placental and fetal "barrier enzyme" 11β-HSD2, which catalyzes cortisol into the biologically less active 11-keto form cortisone (in animals, corticosterone into the inactive form of 11-dehydrocorticosterone). 11β-HSD2 is expressed in the cytotrophoblast in early pregnancy, and increasingly in the syncytiotrophoblast as pregnancy progresses. If glucocorticoid levels exceed the capacity of the enzyme 11β-HSD2 due to increased endogenous production (stress, infection) or exogenous supply (as medication for asthma or autoimmune diseases in pregnancy or for lung maturation induction in the case of threatened premature birth), changes in the HPA axis may occur, which are associated with altered metabolic, cardiovascular and immunological programming.

The expression of the placental enzyme 11β-HSD2 is downregulated by glucocorticoids, maternal stress, hypoxia, and proinflammatory cytokines such as interleukin (IL)-1β or tumor necrosis factor (TNF)-α. Excess of glucocorticoids as well as absence or downregulation of the enzyme 11β-HSD2 result locally in vascular restriction of the fetoplacental circulation and thus in fetal growth restriction (FGR). Thus, continuous therapeutic prednisolone administration in early pregnancy (4^{th}–13^{th} week of gestation) resulted in a doubling of the risk of preterm birth and low birth weight in term infants (Gur et al. 2004). Treatment with pravastatin, which increases vascular endothelial growth factor (VEGF-α) expression in the placenta, improves placental perfusion and may prevent the development of fetal growth restriction in mouse models. Pilot studies of the use of pravastatin to prevent preeclampsia, a condition also associated with angiogenic imbalance, endothelial injury, inflammation, oxidative stress, and thus fetal growth restriction, are ongoing (► Clinicaltrials.gov No. NCT01717586 or Clinicaltrialsregister.eu No. ISRCTN23410175).

In the long term, excess of glucocorticoids and maternal stress seem to result in a hypersensitivity of the HPA axis. The increased reactivity of the HPA axis is reflected in elevated basal cortisol levels and increased responsiveness of the adrenal cortex to ACTH. Maladaptive changes in the HPA axis have been implicated for association with metabolic (insulin resistance), cardiovascular (hypertension, CHD), affective (anxiety disorder, depression), immunologic diseases (asthma, atopic dermatitis, type 1 diabetes mellitus, infections), and cancer (acute lympoblastic leukemia, Hodgkin's and non-Hodgkin's lymphoma, testicular/liver cancer).

Signaling Pathways Via Placentally-expressed Growth Factors

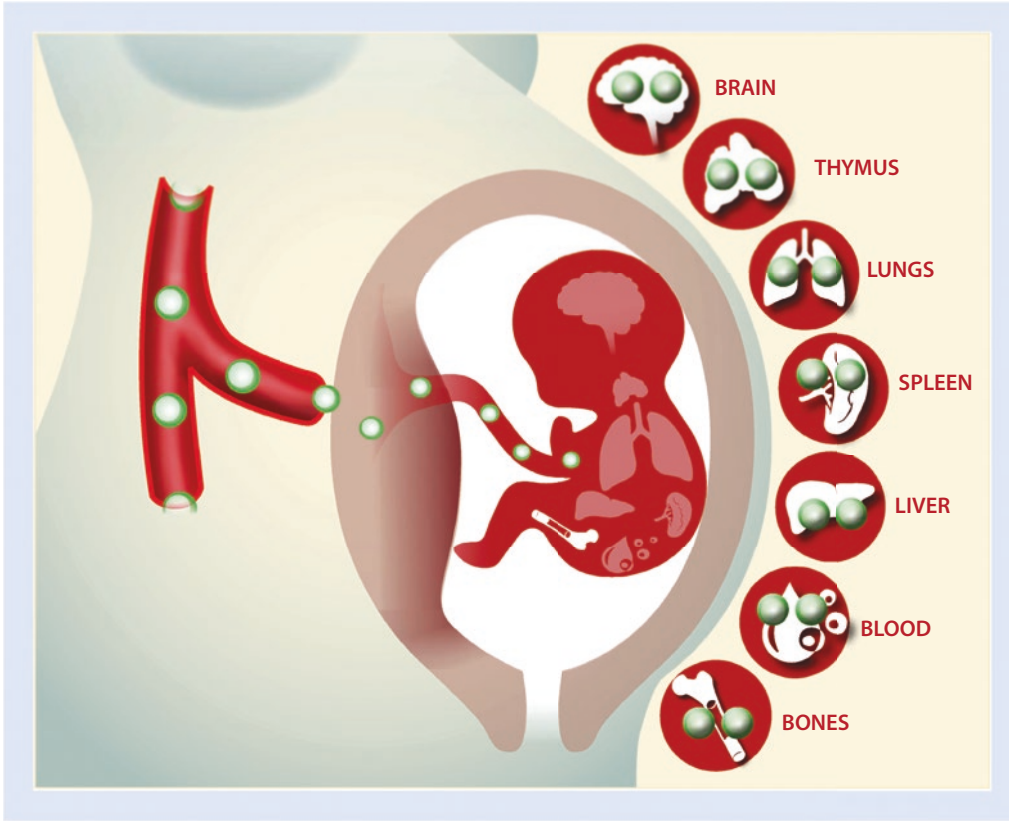
Alterations in the insulin growth factor (IGF) signaling pathway are likely a route by which excessive fetal glucocorticoids can trigger FGR. IGF and their receptors and binding proteins (IGFBPs) are controlled by glucocorticoids. Birth weight inversely correlates with maternal and fetal concentrations of IGF-1 and IGFBP1 (Lassarre et al. 1991). Placental lactogen appears to have a positive effect on glucose, lipid and protein metabolism via stimulation of IGF (Oliver et al. 1992).

Vascular endothelial growth factor (VEGF) is expressed across the amniotic epithelium and cytotrophoblast in pregnancy and is responsible for placental angiogenesis and the development of the villous system in

early pregnancy. Near term, VEGF is responsible for endothelial cell migration and protein synthesis. Glucocorticoids prevent the normal increase of VEGF until term (Hewitt et al. 2006).

Maternal Microchimerism

The bidirectional transplacental exchange of cells between mother and fetus is a physiological process that is accentuated in the second half of pregnancy. If maternal cells are found in the tissues and organs of the fetus, this is referred to as maternal microchimerism (◘ Fig. 13.3). Maternal and thus semiallogeneic cells that have entered the organs of the fetus can sensitize the maturing fetal immune system and can be detected into adulthood (Stelzer et al. 2015). Transplantation immunology has demonstrated that there is tolerance development of the fetal immune system to maternal alloantigens: recipients who received maternal tissue transplantation showed a lower rejection rate, lower graft failure at 6 months and reduced graft-versus-host disease than those who received paternal tissue. One reason for this effect appears to be fetal exposure to non-inherited maternal antigens ("NIMA"), which are transmitted by microchimerism. Induced NIMA-specific regulatory T cells can be detected in the offspring over several generations. Since increased numbers of maternal cells have been found in several autoimmune diseases such as type 1 diabetes



◘ **Fig. 13.3** Maternal microchimerism. Maternal cells cross the fetomaternal blood barrier of the placenta and settle in a variety of fetal organs during pregnancy. These maternal cells, termed microchimeric, can be detected, for example, in the brain, thymus, lung, spleen, liver, and peripheral blood and bone marrow of the fetus and persist long after birth

or Hirschsprung's disease and bronchial asthma, maternal microchimerism is thought to play an important role in the development of these diseases. A direct causal relationship between maternal microchimerism and the development of autoimmune diseases has not yet been demonstrated. However, initial evidence shows that maternal antigens in fetal tissue can induce chronic inflammation by activating fetal T lymphocytes or indirectly control the number of Treg in the offspring.

13.3.2 Epigenetic Changes

Chronic diseases such as diabetes mellitus type 2, CHD or metabolic syndrome show a genetic predisposition. However, epigenetic mechanisms also play a role in the development of diseases: Stimuli/insults of the fetal environment can transform the expression pattern of genes through various types of epigenetic adaptations without changing the base sequence and thus the DNA sequence of the genome per se. Accordingly, it is not the genome alone that determines our traits and characteristics (phenotype), but also how the genome is expressed. These changes can be determined and controlled to varying degrees by intra- and extrauterine influences. Epigenetic modifications can alter gene expression in a stable and long-term manner, and it is discussed that they can be inherited over generations.

Three main processes of epigenetic modification have been identified that determine the extent and timing of gene expression:

- DNA methylation,
- histone transformations by acetylation, methylation, ubiquitinylation, phosphorylation, ADP-ribosylation and
- microRNAs (miRNAs).

These mechanisms interact in a complex interrelated network to precisely regulate gene expression and can influence and alter each other. DNA methylation via cytosine-guanine (CpG) dinucleotides is the most commonly cited epigenetic modification. DNA methyltransferase enzyme isoforms (Dnmt 1, 3a, 3b) methylate DNA and cause a tightly tortuous DNA structure (hyperchromatin) maintained by the heterochromatin protein HP1. Subsequent gene sequences can no longer be read because access of transcription factors to the regulatory elements of the gene is restricted. This leads to a decrease in gene expression and reduced availability of the gene product. Numerous levels of organization, such as the development of receptors, organ development and function, and the organization of hormonal axes, can be regulated via epigenetic changes.

Distinct de- and remethylation processes already take place during a very early phase of gametogenesis and in the preimplantation phase. Epigenetic changes in the trophectoderm from which the placenta develops influence placental development and function. In turn, the placenta is the central organ that helps influence epigenetic regulation of the fetus through altered nutrient transport. For example, the nutrients folic acid, vitamin B12, and methionine can increase the availability of methyl groups. Deficiencies of these nutrients, in turn, can lead to obesity, insulin resistance, and hypertension in the offspring (Gernand et al. 2016). While epigenetic changes have been well studied in animal studies, replicating the results in human studies is challenging: human studies are often limited to studies of cells in the blood, while most epigenetic changes are found in a tissue-specific manner (McDaniell et al. 2010). Some important epigenetic changes will be exemplified below: First, in animal experiments a low-protein diet leads to overexpression of GR and thus increased cortisol sensitivity in liver, kidney, and adipose tissue, as well as decreased expression of the placental enzyme 11-βHSD2. DNA methylations and histone alterations affecting placental expression of glucose transporter 4 (GLUT-4), sodium-coupled neutral amino acid transporter 4

(SNAT-4), peroxisomal proliferator-activated receptor (PPAR)-α and -γ, of interleukin 10 (IL-10), IGF-2, and placental leptin and adiponectin gene regions are implicated as important pathophysiological processes in the development of obesity and insulin resistance (Raychaudhuri et al. 2008; Simmons 2007; Tobi et al. 2009; Bouchard et al. 2012).

DNA methylation levels are generally low and the extent is estimated to be only about 5% in stress-related genes, for example (Non et al. 2012), so the biological significance is unclear. In fact, few studies have examined these small differences in DNA methylation levels for their functional consequences.

13.4 Examples of Exogenous Stimuli of Fetal Programming

13.4.1 Maternal Malnutrition and Placental Insufficiency

It is generally accepted that maternal diet particularly affects fetal health. In animal studies, general food restriction leads to hypertension, vascular dysfunction, increased appetite, obesity and impaired glucose tolerance. Specifically, maternal malnutrition with a low-protein diet appears to be associated with the occurrence of hypertension, increased angiotensin-converting enzyme (ACE) activity, a reduced number of nephrons, and oxidative stress in adulthood of the offspring (Watkins et al. 2011). The consequences of protein restriction are most severe when it begins at the end of pregnancy. Low-protein diet-induced hypertension in rats can also be prevented by metyrapone, an inhibitor of maternal glucocorticoid synthesis (Langley-Evans 1997). Glucocorticoid therapy during pregnancy has long been suspected of being responsible for low birth weight and chronic disease in adulthood (Sect. “Hypothalamic-pituitary-adrenal axis”). In animal experiments, low birth weight and persistent elevation of blood pressure in adult offspring can be induced by malnutrition in rats. This effect could be induced by carbenoxolone, an inhibitor of the placental enzyme 11β-HSD2. 11β-HSD2 appears to play a key role in the increased placental delivery of endogenous maternal glucocorticoid to the fetus, a mechanism that appears to underlie the consequences of a low-protein diet. Thus, 40–50% dietary restriction results in increased maternal and neonatal glucocorticoid levels in animal studies (Lesage et al. 2001). A reduction in the placental enzyme 11β-HSD2 can be found not only in maternal malnutrition, but also in maternal stress, hypoxia and vitamin D deficiency (Tesic et al. 2015).

At the placental level, the nutrient-sensitizing signaling pathway via the serine/threonine protein kinase (“mammalian target of rapamycin”, mTOR) appears to be downregulated in FGR, which in turn decreases placental transport of L-amino acids, folic acid, and oxygen (Jansson et al. 2012; Rosario et al. 2016). In the low-protein diet model, decreased expression of the mTOR complex 1 (mTORC1) has been shown in rats and monkeys, consistent with human studies in FGR (Chen et al. 2015). A recent review deals in detail with placental nutrient transporters and possible influences (Braun et al. 2013).

An adequate placental supply of long-chain polyunsaturated and omega-3 fatty acids to the fetus appears to be important not only for the normal development of the brain and cardiovascular system, but these also ensure normal placentation and have antioxidant and anti-inflammatory properties. Omega-3 fatty acid supplementation in pregnancy appears to have beneficial effects on brain development, for example, but does not improve the incidence of FGR and hypertensive diseases during pregnancy in high-risk constellations (Horvath et al. 2007). One reason for this is seen in the

placental deficiency supply and not in the maternal supply.

In the animal model of "artificial" placental insufficiency, hypoxia is induced by direct occlusion of vascular perfusion. Ligation of a uterine vessel results in morphological changes of the heart (increased fibrosis), the aorta (thickened wall) and the kidneys (increased fibrosis and decreased nephron number) of the fetus (Briscoe et al. 2004). The growth retarded juveniles are chronically hypoxic, hyperglycemic and show altered brain development.

13.4.2 Maternal Oversupply and Gestational Diabetes

Transplacental glucose exchange occurs via isoforms of the glucose transporter (GLUT) family and follows the glucose gradient passively from mother to child (Desoye and Nolan 2016). Glucose uptake via placental glucose transporters can be stimulated by insulin in the first trimester. However, the expression of glucose transporters at term cannot be influenced by insulin, leptin, insulin-like growth factor (IGF) and growth hormone (GH). Maternal hyperglycemia may thus reach the fetus increasingly unaffected during the course of pregnancy. The pancreas and liver are induced by increased glucose, lipid and amino acid levels to increase the secretion of insulin and IGF-1. Placental IGF-2 in particular is stimulated, which leads to an increased growth rate of the child (fetal macrosomia). In line with the picture in FGR, activation via the mTOR pathway can be found in gestational diabetes (GDM) (Capobianco et al. 2016). Low grade inflammation seems to be a crucial link between insulin resistance, obesity and type 2 diabetes mellitus. Adipokines and cytokines alter insulin sensitivity by affecting the insulin signaling pathway and induce inflammation. Adiponectin is produced by adipose tissue and enhances the action of insulin at end organs, has anti-atherogenic and anti-inflammatory effects. Women with gestational diabetes secrete reduced levels of adiponectin due to increased levels of inflammatory TNF-α and IL-6, which curb the expression of adiponectin (Atègbo et al. 2006). Leptin produced in the placenta and adipocytes has appetite regulating effects, is proinflammatory and regulates lipid metabolism. Mothers with GDM have increased leptin levels, but the offspring have reduced leptin levels. Leptin deficiency appears to promote the development of obesity.

13.4.3 Glucocorticoid Administration for Lung Maturation Induction

The corticosteroids beta- or dexamethasone administered for lung maturation in threatened preterm birth are not inactivated by the enzyme 11β-HSD2, bypass the fetoplacental barrier unimpeded and are 25- to 30-fold more potent than cortisol in the fetal body system. The synthetic glucocorticoids act as agonists of the glucocorticoid receptors, with dexamethasone having greater affinity than betamethasone (Kapoor et al. 2008). They inhibit the fetal HPA axis in a negative feedback mechanism for at least 1 week. Most studies of the possible influences of glucocorticoid therapy given antenatally include preterm infants and are suspected of often inadequately accounting for confounders such as prematurity, related comorbidities, and neonatal intensive care. Term infants whose mothers had received lung maturation induction during pregnancy show significantly elevated cortisol levels in response to a pain-inducing plantar stitch (Davis et al. 2011). Another study that followed up 209 term-born children aged 6–11 years confirmed these findings, demonstrating increased cortisol secretion to acute psychosocial stress in children (more so in girls) whose mothers had received glucocor-

ticoids prenatally, as evidence of increased HPA axis responsiveness (Alexander et al. 2012). Prenatally applied glucocorticoid therapy is also suspected to promote later asthma (Sect. "Hypothalamic-pituitary-adrenocortical axis"). Dexa- and betamethasone seem to negatively influence the function of neutrophils and T-lymphocytes (Veru et al. 2015).

Tip

Both the initial indication and a repeat of lung maturation induction should take into account the possible long-term consequences for the offspring.

13.5 Contradictions and Alternative Approaches

Obviously, there are a vast number of factors influencing the later development of health and disease that are not solely based on genetic predisposition and life changes. There are many possibilities for adaptive processes at the cellular level as well as in cytokine and hormone signaling pathways. Variants of the genetic pool as well as epigenetic changes and also the interaction of gene variants and the epigenome influence the outcome of the offspring. Observational studies associating birth weight with metabolic, cardiovascular, or psychiatric diseases often fail to duly incorporate genetic factors. An alternative hypothesis to FOAD is the presence of gene variants associated with chronic diseases: E.g., a strong genetic component underlies type 2 diabetes mellitus. According to the "fetal insulin hypothesis", any gene variant that reduces insulin secretion also results in a decrease in birth weight, since fetal growth is dependent on insulin. Thus, by examining single-nucleotide polymorphisms (SNPs) of two different gene loci, CDKAL1 and HHEX-DIE, an association of fetal genotype with both low birth weight and increased risk of type 2 diabetes mellitus has been shown (Freathy et al. 2009).

13.6 Pregnancy as an Option for Future Health Prevention

Unfortunately, in the discussion of the concept of FOAD, there is often a thoughtless attribution of blame in maternal behavior. Initial studies suggest that a healthy diet, regular physical activity and stress reduction measures during pregnancy can have a positive influence on pregnancy outcomes and also on the health of the offspring (Urizar et al. 2004).

However, there is currently too little research to integrate revolutionary interventions into prenatal care. Thus, most recommendations refer only to instruction in a healthy balanced diet and regular exercise during pregnancy. The significance of FOAD has long been recognized by WHO and in 2012 WHO published recommendations on maternal, infant and child nutrition with the aim of reducing the prevalence of low birth weight.

Factors such as paternal influence (e.g. pre-conceptional nutrition and stress, etc.), family life and the social environment still receive too little attention. For example, the influence of paternal nutrition and stress on the later development of chronic diseases is insufficiently studied. The complexity of the underlying pathomechanisms should not be ignored. Thus, a number of genetic, socioeconomic, lifestyle, and environmental factors that have received little attention and have rarely been studied to date may also contribute to risk. Many stressors that influence disease risk are rooted in social class, ethnicity, and gender roles, and sometimes require societal changes, not just individual interventions. The findings on FOAD should not lead to pathologizing pregnancy,

but should be used to identify and eliminate any suboptimal factors as early as possible, so that the pregnancy can take the least stressful course possible.

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Fetal Cells and Cell-Free Nucleic Acids in Maternal Blood: Genetic and Immunological Aspects

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Contents

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14.1 Introduction

A decisive breakthrough in prenatal health care came with the development of noninvasive tests for the detection of fetal aneuploidies, such as those for trisomy 21, the cause of Down syndrome (Lo 2015; Hui and Bianchi 2017). These tests are based on the detection of cell-free fetal DNA molecules in samples of maternal blood (Lo 2015).

Although the translation of this technology from the laboratory bench to daily use in the clinic has been extremely rapid, the journey leading to this development has been long and arduous and involved the analysis of a number of different sources of fetal genetic material (Lo 2015).

While the cell-free genetic material used in tests today is derived from the placenta, previous studies have used circulating trophoblast cells released from the placenta or fetal blood cells that have crossed the placental barrier (Holzgreve and Hahn 2000). Another potential source of fetal genetic material that has already been studied is the presence of trophoblast cells in the cervical smear (Holzgreve and Hahn 2000).

Today, it appears that some of these early approaches, which were not pursued further, are regaining interest as they allow analyses that are not possible with fragmented cell-free DNA delivered from the placenta (Drewlo and Armant 2017).

Another caveat to the rapid transfer of technology is that the underlying biology and potential consequences of this fetomaternal exchange of cells and genetic material are readily forgotten, especially in light of the fact that financial support in this research field is already declining after the technology has already been successfully implemented in the clinic (Hahn et al. 2014, 2005).

In this chapter, we will review the history, current status, and unforeseen developments that will potentially affect a wide range of areas, including delivery, prevention of infection, and the development of autoimmune diseases after birth.

14.2 Fetal Cells and Fetal DNA in Maternal Blood: A Centuries-Old Phenomenon

Those who are unfamiliar with history put themselves in danger of repeating it; while those who have a fundamental knowledge of past events are aware of the path towards new developments. In this regard, the first documented description of fetal cells entering the maternal circulation was made by the German pathologist Georg Schmorl in the late 1890s, when he found placental trophoblast cells in the lungs of women who had died of eclampsia (Lapaire et al. 2007). Evidence of other fetal cells that may have entered the maternal bloodstream was initially obtained indirectly in cases with hemolytic disease of the newborn ("HDN") (Hahn et al. 2005). The disease was first described in the 1930s when it became clear that the disease occurred in pregnant women who lacked the rhesus D gene.

The discovery that the rhesus D antigen is not expressed on the erythrocytes of these women led to the conclusion that fetal erythrocytes must have crossed the placental barrier. Since this antigen is not expressed on rhesus D-negative cells, the rhesus D-positive erythrocytes stimulated a distinct immune response in the mother, which can be fatal for the fetus.

The development of a stain for the detection of fetal hemoglobin was achieved by Enno Kleihauer and Klaus Betke (Kleihauer-Betke stain). This stain provided the final evidence for the presence of fetal erythrocytes in samples of maternal blood (Holzgreve and Hahn 2000). This simple chemical stain became a valuable tool in testing rhesus D negative women who were at increased risk for HDN or other conditions associated with fetomaternal bleeding. This stain is still used for this purpose in many centers worldwide.

A technological quantum leap that significantly advanced the field was the invention of flow cytometry and fluorescent activated cell sorting (FACS) by Leo Herzenberg and colleagues (Holzgreve and Hahn 2000). Because this new method allowed detection and sorting of rare cells, it was quickly adopted by those looking for rare fetal cells in maternal blood. Among these individuals was Diana Bianchi, who stood out as a leader in the field, shaping the field for decades and driving solid transfer to clinical reality (Bianchi and Wilkins-Haug 2014). With her new leadership role as Director of the NICHD (National Institute of Child Health and Development) at the NIH (National Institutes of Health) in the US, her guidance and advocacy for the field will remain.

A decade ago, the erythroblast was the fetal cell of choice. This was due to its abundance in fetal blood, its short half-life (about 30 days), its genetic integrity and stability (not susceptible to mosaicism) and the availability of suitable antibodies for enrichment and detection (fetal hemoglobins) (Holzgreve and Hahn 2000).

In addition to FACS in the USA, similar investigations were advanced in Europe with the technology MACS ("magnetic cell sorting"). Both systems (FACS and MACS) have been used in parallel in a variety of multicenter studies, for example, the NIH-funded NIFTY fetal cell isolation study and the EU-funded SAFE (Saline vs. Albumin Fluid Evaluation) study. Interestingly, these studies found that neither system was suitable for use in the clinic.

At the same time, there were investigations by other research groups as to whether the use of transcervical cells would be suitable (Holzgreve and Hahn 2000). However, these studies were discontinued, mainly because it was shown that the collection of the cells was an invasive procedure associated with an increased risk of abnormal development of the fetal limbs.

During the final phase of the NIFTY study, Dennis Lo et al. in Oxford discovered that cell-free fetal DNA could be detected in maternal plasma and serum using polymerase chain reaction (PCR) (Lo 2015). This analysis had been inspired by reports that certain tumor patients had high levels of cell-free tumor DNA in their blood (Lo 2015). The reasoning derived from this was that the placenta shares some characteristics with tumors, such as high cell turnover or proangiogenic vascular activity. Therefore, Dennis Lo investigated whether the placenta—similar to tumors—releases DNA fragments into its environment. This led to a completely new way of prenatal genetic analysis (Lo 2015).

Initially, this approach was used to provide simple detection of genetic loci of the fetus that are completely absent from the maternal genome, such as the Y chromosome in a male fetus or the Rhesus D gene in Rhesus D negative females. This was quickly extended to other hereditary diseases that follow Mendelian laws, especially hemoglobinopathies, which required the detection of complex genetic targets such as point mutations or small deletions (Lo 2015).

The approach of measuring fetal DNA in maternal blood was so successful that it was quickly adopted by almost all research groups in the field (Lo 2015). The procedure for blood samples changed accordingly: While previously the plasma fraction of a maternal blood sample had been disposed of in order to access the rare cells in the cell pellet ("buffy coat"), now the cellular fractions were disposed of and the plasma fraction was used.

As will be discussed in detail later, cell-free DNA analysis was able to achieve the final goal of noninvasive prenatal diagnostics, namely the detection of chromosomal abnormalities in the fetus, through the use of massive parallel sequencing, appropriate algorithms, and the necessary computing capabilities (Lo 2015; Bianchi and Wilkins-Haug 2014).

Once again, as with so many developments in the field, it is ironic to see that the presence of nucleic acids in the extracellular milieu was already described by Mandel and Métias in 1948, long before Watson and Cricks published their sensational discovery of the structure of DNA (Lo 2015). So it seems that the wheel of time has to turn a lot until we eventually become aware of something.

14.3 Cell-Free DNA: Current Status for Non-invasive Prenatal Testing

The ability to obtain fetal genetic information from maternal blood has been a long-sought goal in prenatal medicine (Lo 2015; Hui and Bianchi 2017). The detection of fetal DNA in maternal blood, coupled with the development of powerful next generation sequencing (NGS) techniques enabled the transfer of this analysis into clinical practice (Lo 2015; Hui and Bianchi 2017; Hahn et al. 2011). Over the past 10 years, NGS has evolved from the laboratory bench into clinical laboratory diagnostics (Lo 2015; Hui and Bianchi 2017; Hahn et al. 2011).

In addition, the commercial introduction of non-invasive prenatal testing (NIPT) for the detection of aneuploidies has created a very strong demand. This in turn drove the extremely rapid development and improvement of technologies (Lo 2015; Hui and Bianchi 2017). Scientific publications in this field are now so numerous that it is a challenge to keep up to date.

In fact, since the first company offered the first analysis for trisomy 21 in the US in 2011, this method has been developed extraordinarily quickly. The reason was the great need for risk-free genetic prenatal testing. Today, this method can also be used to detect other aneuploidies (Lo 2015; Hui and Bianchi 2017).

All currently available non-invasive prenatal tests for fetal aneuploidies are based on the detection of cell-free DNA in maternal blood. The presence of fetal DNA in maternal blood can be explained by the continuous release of large amounts of cell-free nucleic acids by the placenta. However, it must be emphasized that only about 10% of the circulating cell-free DNA is of fetal origin, while about 90% is derived from maternal sources. Fetal cell-free DNA is found in maternal blood only during pregnancy and is no longer detectable a few hours after birth.

Today, three basic approaches are commercially available to detect aneuploidies in maternal blood:

- Whole Genome Sequencing,
- Targeted Genome Sequencing and
- Sequencing based on single nucleotide polymorphisms (SNP) (◘ Table 14.1) (Lo 2015; Hui and Bianchi 2017).

All approaches use Massive Parallel Sequencing (MPS) or Next Generation Sequencing (NGS). These terms refer to high-throughput DNA sequencing methods that allow millions of DNA molecules to be sequenced in a single reaction (in parallel). All three approaches also have in common that the sequenced DNA material comes from both the mother and the fetus.

14.3.1 Whole Genome Sequencing

For whole genome sequencing, the entire fragmented cell-free DNA is used as a template and sequenced in short repeats. Then, each fragment is reassembled through a complex bioinformatics comparison that uses a database of the human genome to assign fragments to specific chromosomes (Lo 2015). If a fetus has a supernumerary chromosome (as in trisomy 21, 13, or 18), there should be more fragments from that chromosome compared to a normal karyo-

Table 14.1 Selection of different non-invasive prenatal tests (NIPT)

Company	Name	Test Offer	Offered from week of gestation	Method	Duration (days)	Twins
Ariosa Diagnostics (USA)	Harmony®	Trisomy 21, 13, 18, Gonosomal aneuplodies	10 + 0	Chromosome selective sequencing	9–14	Yes
Genesupport (CH)	Prendia®	Trisomy 21, 13, 18, Gonosomal aneuplodies, Structural chromosomal anomalies and rare autosomal trisomies	9 + 0	Whole genome sequencing	15	Yes
Lifecodexx (D)	PraenaTest®	Trisomy 21, 13, 18, Gonosomal aneuplodies	9 + 0	Whole genome sequencing	10–14	Yes
Natera (USA)	Panorama®	Trisomy 21, 13, 18, Gonosomal aneuplodies Triploidies, Existing microdeletions	9 + 0	SNP targeted sequencing	9–12	No

SNP single nucleotide polymorphism

type (Fig. 14.1). However, it is necessary to sequence many millions of DNA fragments from the mix of maternal and fetal cell-free DNA (12–25 × 10^6 assigned sequences). This is necessary to ensure that there are sufficient chromosome fragments for the particular chromosome in question to represent statistically significant differences between aneuploid and euploid fetuses in this complex analysis.

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14.3.2 Targeted Genome Sequencing

Since the approach described above is neither time- nor cost-efficient, in contrast to whole-genome approaches, targeted approaches have been developed that specifically target the region or chromosome of interest (Lo 2015; Hui and Bianchi 2017). The idea is to selectively amplify specific regions from the chromosome of interest (e.g., chromosome 21), followed by massive parallel sequencing (MPS). This method is also called digital analysis of selected regions (DANSR). With this technique, the amount of DNA to be sequenced (Fig. 14.2) is much smaller than with the whole-genome approach to detect a specific aneuploidy (between 40,000–10^6/sample). The commercially available test using the targeted approach together with MPS then needs a risk score of trisomy evaluation optimized for the fetal fraction of DNA ("fetal fraction optimized risk score of trisomy evaluation", FORTE) to perform a risk calculation for the individual chromosomal aberration. FORTE is an algorithm that takes into account the a

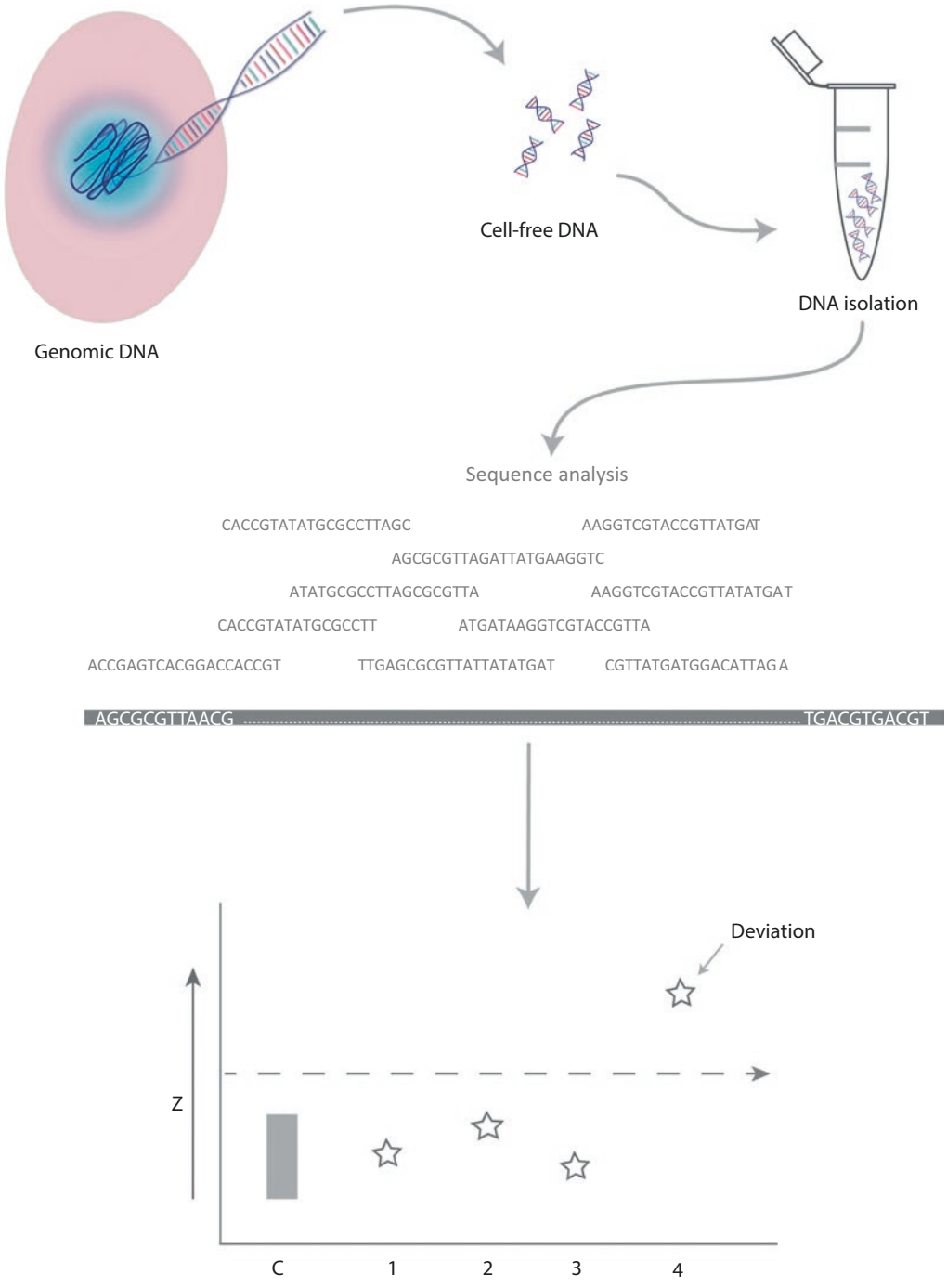

Fig. 14.1 Schematic of the sequencing procedure used to detect fetal aneuploidies by analysis of cell-free DNA

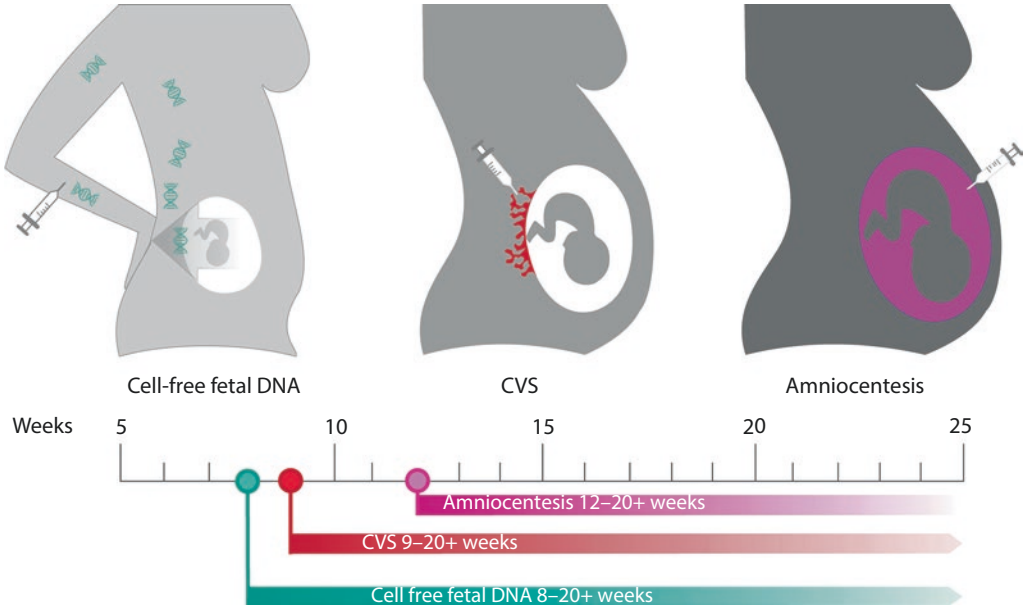

Fig. 14.2 Analysis of cell-free DNA allows earlier detection of fetal anomalies than invasive methods such as chorionic villus sampling (*CVS*) or amniocentesis

priori risk (maternal age, week of gestation) and uses a probability approach (odds ratio) to calculate the risk of aneuploidy.

14.3.3 SNP Approach

This third approach is the newest on the market and was first offered in December 2012 (Lo 2015; Hui and Bianchi 2017). The test is based on the amplification of 11,000 SNP sequences, which represent the most common type of genetic variants between single individuals and refer to a single different base pair in a DNA segment. This approach performs allele ratio analysis and requires supporting data from the parents (e.g., maternal leukocytes from a maternal plasma preparation) for analysis of the targeted MPS data. Both maternal DNA and the mixture of maternal and fetal DNA are sequenced separately. Then, the fetal signal is read from the difference between pure maternal DNA and the mixture of maternal and fetal DNA. Each result for each chromosome is considered against the background that the fetus is monosomal, disomal or trisomal. The analysis calculates different probability distributions because for each allele a set of SNPs is expected on the target chromosome. This is done depending on the maternal genotype, the fetal portion of the DNA, and the fetal chromosome number. The laboratory methods and data analysis used for the SNP-based NIPT approach are substantially different from other approaches (such as shotgun or targeted counting). In addition, they cannot be used in pregnancies where the mother is not genetically related to the fetus, such as egg donation.

Since this method analyzes allele distribution and does not require a disomal reference chromosome, it is expected to be particularly useful in detecting triploidy.

14.3.4 Technical Principles of Currently Available Non-invasive Prenatal Tests

The sensitivity of cell-free DNA testing in clinical trials is >99% for trisomy 21 (with a false-positive rate of 0.1–0.4%), about 98% for trisomy 18, and about 92% for trisomy 13. The positive predictive value as a basis for counseling for positive results depends on the prevalence of the condition and is not reported for most available NIPT tests. Here it can be assumed that these tests want to seem more diagnostic than they are.

14.3.5 Clinical Use of NIPT, Its Limitations and Impact on Prenatal Care

National and international societies have recommended noninvasive prenatal testing for high-risk pregnancies after preconception counseling (Hui and Bianchi 2017; Hahn et al. 2012a). The challenge for physicians will be to counsel pregnant women about which test to use for which indication. Here, a cautious approach that incorporates ethical considerations is of utmost importance. Only in this way can these powerful methods be used sensibly and sensitively (Hui and Bianchi 2017; Hahn et al. 2012a).

Non-invasive prenatal testing is usually used as a second test after first trimester screening.

The main advantage of NIPT is the reduction of invasive procedures. Therefore, models for the use of these tests as screening tests are now emerging. On the other hand, the main disadvantage is the significant number of tests that do not reach the quality criteria. This is mainly due to a very small proportion of fetal cell-free DNA, affecting up to 5% of all pregnant women. Other limitations of this method include the need for a subsequent invasive test if the NIPT was positive, or the turnaround time of about 5 days compared to invasive tests, which can provide preliminary results within 24 h.

The great challenge today is to develop algorithms for the prenatal care of women who have opted for prenatal testing. For this to happen, non-invasive prenatal testing must be inserted into the relevant guidelines to allow general access that is financed by health insurance companies. This must apply to all women who would genuinely benefit from this NIPT in terms of public health (Hui and Bianchi 2017).

14.4 Trophoblast Cells in the Cervix – Diagnostic Use and Insight into Fetomaternal Pathologies

Distinct cell types with unique functions are required in the placenta to ensure a successful pregnancy (Burton and Jauniaux 2015). One of these is the trophoblast. Trophoblast cells can be divided into two main lineages: the villous trophoblast and the extravillous trophoblast.

The villous trophoblast lineage develops an elaborate cellular network that shapes the surface of the villous tree. This surface structure comes into direct contact with maternal blood and serves to absorb nutrients, exchange gases, and dispose of waste products from the growing fetus. As pregnancy progresses, the syncytiotrophoblast releases apoptotic material into the maternal circulation. It also releases exosomes that further communicate their contents. These include microRNA molecules that target various maternal organ systems.

Extravillous trophoblast cells distribute starting from the placental anchoring villi and invade the maternal decidua. Thus, they generate an interface between the placenta and the uterine wall. In addition, they allow the flow of maternal blood into the intervillous space of the placenta. As the extravillous trophoblast cells move away from the

implantation site, they differentiate into distinct cell types that can be distinguished from each other based on their localization and marker expression (Burton and Jauniaux 2015).

For more than 40 years, researchers have been searching for alternatives to amniocentesis to develop effective and accurate methods for noninvasive diagnosis (Lo 2015; Hui and Bianchi 2017; Holzgreve and Hahn 2000). Three main techniques have emerged in the last 40 years:

- the use of fetal cell-free DNA in maternal blood,
- the enrichment of fetal erythroblasts from the blood of pregnant women and
- the collection of trophoblast cells from the cervical region.

The latter technique has recently been revised and now shows promising results (Drewlo and Armant 2017). It is presented in more detail here.

The presence of trophoblast cells in the reproductive tract has been known for many years. However, the lack of methods to isolate these cells in sufficient numbers and purity for unequivocal analysis prevented their use in clinical tests and in biological studies (Holzgreve and Hahn 2000).

How extravillous trophoblast cells reach the reproductive tract and especially the cervix is still not fully understood (Drewlo and Armant 2017). Today, there are two main hypotheses that are being discussed:

1. The first hypothesis postulates that extravillous trophoblast cells migrate radially away from the embryo in early pregnancy and break through the decidua capsularis.
2. The second hypothesis is based on the knowledge that secretory products of the endometrial glands support placental growth and development during the first 10 weeks of pregnancy, before maternal blood flows into the intervillous space.

Studies have shown that extravillous trophoblast cells actively invade uterine glands to allow them to communicate directly with the intervillous space of the placenta (Moser et al. 2010, 2017). This invasion pathway allows for a hypothesis with the following assumption: extravillous trophoblast cells invaded into the lumen of a uterine gland at the edge of the placenta are flushed into the uterine cavity by the transport of the secretory products of the gland and migrate from there to the cervical canal. This endoglandular trophoblast could be a constant source of placental cells that pass through the cervical canal during the first half of pregnancy. During this time, they can be successfully retrieved and isolated from the cervix ("trophoblast retrieval and isolation from the cervix", TRIC) (Drewlo and Armant 2017).

Over time, the identification of fetal cells has been achieved by different approaches. The first method used a combination of morphological identification and micromanipulation to isolate clusters of cells that looked trophoblast-like. The second and most recent method used the "Cytobrush," a brush used for cervical gynecological smears. Further use of the anti-HLA-G antibody G233 then allows imaging of HLA-G-positive extravillous trophoblast cells and isolating cellular elements that could be extravillous trophoblast cells with laser microdissection (Drewlo and Armant 2017; Moser et al. 2010).

Other trophoblast markers have been applied such as various cytokeratins and antigens expressed by villous and extravillous trophoblast cells. Among the various cytokeratins, cytokeratin 7 has emerged as the most useful marker for all human trophoblast phenotypes (Drewlo and Armant 2017).

The TRIC method can be used to obtain endocervical samples containing extravillous trophoblast cells between the fifth and 20th week of gestation. These samples are fixed in a transport medium and extravillous

trophoblast cells are isolated via HLA-G-MACS (Drewlo and Armant 2017). The extravillous trophoblast cells can be mounted on slides or frozen in tubes for RNA or DNA isolation. Trophoblast-like cells, as determined by immunofluorescence with an antibody against beta-hCG, have an average purity of 89.2%. This value correlates well with the proportion of fetal DNA in these isolates, which—based on sequencing data—is 92.2%. The genotype of the cervical trophoblast-like cells correlates 100% with the placental cells and is different from, but related to, the maternal genotype.

Isolation of fetal cells by transcervical collection is considered a non-invasive method for prenatal diagnosis of chromosomal aberrations of the fetus. The standardization of this clinical method is not yet completed.

The frequency of trophoblastic cells in transcervical specimens and their identification by immunohistochemical staining with HLA-G antibodies may pave the way for this noninvasive method to be used as well for the differentiation of pathological and normal pregnancies.

On this topic, Drewlo et al. investigated whether HLA-G-positive trophoblast cells isolated by TRIC can be used as a proxy for placental function (Drewlo and Armant 2017). The basis of this research was the effect that altered differentiation of extravillous trophoblasts has on syndromes of placental insufficiency. As an approach to this analysis, the authors used immunofluorescence and single-cell image analysis for a semiquantitative evaluation of seven proteins that exhibit altered serum levels in women with placental insufficiency. Significant differences were measured in the expression of PAPP-A, FLT1, ENG, AFP, PlGF, and LGALS14, but not LGALS13 or the trophoblast marker KRT7.

These data form the basis for new research projects aimed at developing new approaches to detect placental dysregulations already in early pregnancy (Drewlo and Armant 2017). However, it should be noted that trophoblast-rich cervical mucus from the first trimester of pregnancy has so far been used primarily for genetic studies.

14.5 Placental Cell-Free DNA: An Activator of the Maternal Immune System and Initiator of Birth?

The mechanisms regulating birth induction are still unclear (Phillippe 2014). The discovery of cell-free DNA in maternal blood opened the possibility of finding new modulators of labor induction (Hahn et al. 2014; Phillippe 2014). Fetal cell-free DNA found at term is predominantly based on DNA released from the placental trophoblast via apoptotic or necrotic processes. Evidence for an influence of fetal cell-free DNA on labor induction comes from data showing that there is a marked increase in the concentration of fetal cell-free DNA in maternal blood toward the end of pregnancy (Hahn et al. 2014, 2005). A possible link between fetal cell-free DNA and induction of labor is also supported by data showing that women who experience preterm birth also have significantly increased concentrations of fetal cell-free DNA in maternal blood compared to the control group (Hahn et al. 2014, 2005).

It is generally accepted that spontaneous parturition is initiated by inflammatory processes triggered by mechanisms unrelated to the presence of exogenous pathogens (Romero et al. 2014). To date, proinflammatory factors responsible for inflammatory onset in pathogen-free conditions are not known. Fetal cell-free DNA may be a promising linking element in the relationship between sterile inflammation and birth induction (Hahn et al. 2014; Phillippe 2014; Scharfe-Nugent et al. 2012). There is already first evidence that fetal cell-free DNA may be one of the inflammatory triggers. In the cell,

DNA is well packaged in the nucleus and mitochondria. This DNA is safe and protected from interactions with immune system DNA sensors. Once the DNA is released from the nucleus or mitochondria into the cytoplasm of the cell or even into the extracellular space, it could potentially become so-called Danger Associated Molecular Patterns (DAMPs). These DAMPs are recognized by the immune system.

In addition, DNA derived from the placental trophoblast has a unique phenotype that makes it even more immunostimulatory than somatic DNA. Trophoblast DNA has many non-methylated cytosine-phosphate-guanine (CpG) islands. Thus, it resembles bacterial DNA, which is highly inflammatory. Hypomethylated cell-free DNA is recognized by pattern-recognition receptors, such as Toll-like receptor 9 (TLR9), which recognizes hypomethylated CpG. Animal studies have shown that activation of TLR9 during pregnancy induces preterm birth (Scharfe-Nugent et al. 2012). Injection of fetal cell-free DNA into pregnant mice causes fetal resorption, increased levels of tumor necrosis factor alpha (TNF-alpha) and IL-6, and stimulates infiltration of inflammatory cells into the placental bed. This effect was not found when cell-free DNA of adult origin was injected.

The importance of the TLR9 receptor was further supported by experiments with TLR9 knockout mice. Here, fetal cell-free DNA had no effect on pregnancy. In human in vitro studies, fetal DNA activated the transcription factor NF-Kb, followed by the production of IL-6 in human B cells and peripheral blood mononuclear cells from both pregnant and nonpregnant donors. These experiments support the hypothesis that fetal DNA triggers an immune response via the TLR9 receptor and thus may play a role in birth induction. A lack of balance in these processes could contribute to triggering preeclampsia or preterm birth.

However, it must be made clear that direct evidence is still lacking. Experiments with DNA from human umbilical cord blood injected into pregnant mice failed to show a sufficient inflammatory response. Arguments against an inflammatory efficacy of fetal DNA were also provided by a study (Conka et al. 2017) that could not reproduce any of the above effects of human or mouse DNA despite similar experimental conditions. Based on the negative results, the authors of this study concluded that the increase in fetal cell-free DNA in pregnancy-associated diseases is a consequence rather than a cause.

Nevertheless, fetal DNA has a very unique epigenetic profile that changes depending on conditions during pregnancy. Extensive hypomethylation of trophoblast DNA has been found in preeclamptic placentas compared to controls (Hahn et al. 2014, 2005). To date, it is unclear whether there are changes in inflammatory capacity in relation to hypomethylation status. This correlation needs further investigation. A further limitation of the results arises from the animal model used, as preeclampsia does not occur in mice during pregnancy.

14.6 MicroRNA from the Placenta: New Antiviral Agents?

Severe pathologies and diseases can derive from vertical viral transmission from mother to fetus, especially those such as Zika virus (Rather et al. 2017). Therefore, strategies to reduce infections are essential. In addition, innate antiviral immunity at the fetomaternal interface is of great importance. Here, the trophoblast is critical for preventing the spread of pathogens, such as viruses to the fetus (Burton and Fowden 2015). In addition to limiting viral access to the fetus through mechanical and immunological barriers, the trophoblast has evolved other strategies to reduce maternal susceptibility to viral infections (Burton and Fowden 2015).

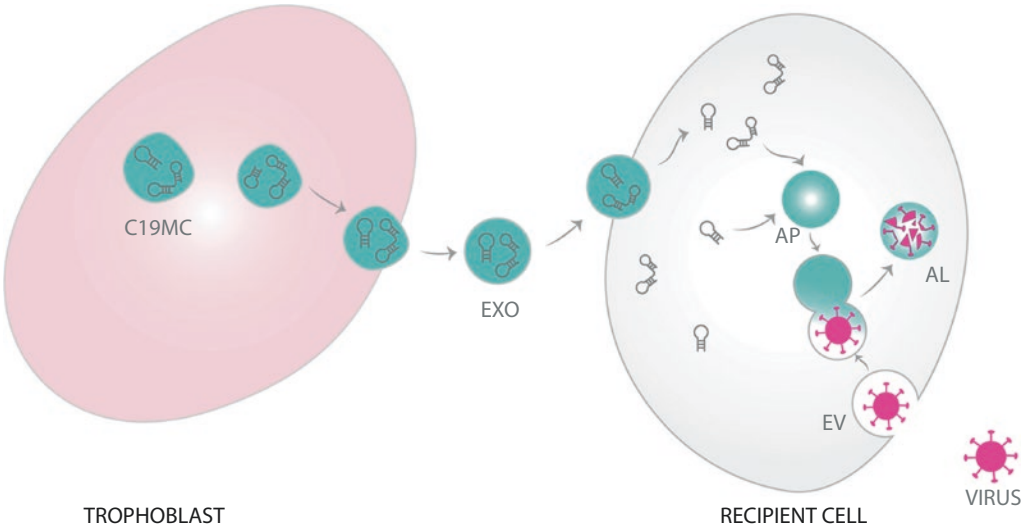

Fig. 14.3 Schematic representation of how miRNA from chromosome 19 is packaged into trophoblast exosomes and protects maternal cells from viral infection. *AP* autophagosome; *AL* autolysosome, *EV* endocytic vesicle; *EXO* exosome; *C19MC* microRNAs from chromosome 19

MicroRNAs (miRNAs) are small non-coding RNA molecules about 22 nucleotides in length that are found in all eukaryotes (except fungi) (Dumont et al. 2017). In general, miRNAs can regulate gene expression by destabilizing mRNA and by "translational silencing". During pregnancy, the trophoblast expresses miRNAs derived predominantly from the chromosome 19 miRNA cluster (C19MC) (Morales-Prieto et al. 2013). This cluster spans over 100 kb of genomic DNA, is epigenetically controlled, contains 46 intronic miRNA genes flanked by Alu repeats, and 37 highly repetitive short DNA segments (+/−120 nucleotides) that may be spliced exons of non-coding short-lived RNA transcripts whose function is as yet unknown (Morales-Prieto et al. 2013).

Delorme-Axford et al. (2013) have shown that exosomes of the primary human trophoblast are loaded with placenta-specific C19MC miRNA molecules and influence the maternal immune response to viral infections. The following observations were made in this study: (1) Primary trophoblast cells are resistant to infection by a variety of unrelated DNA and RNA viruses. (2) When primary trophoblast cells were cultured in vitro, their viral resistance could be transferred to non-placental cells. These included physiologically important maternal and other primary cells when incubated with culture medium from primary trophoblast cells. (3) C19MC-miRNAs induce autophagy in non-placental cells, thereby increasing antiviral defense. (4) C19MC-miRNAs are found in exosomes of primary trophoblast cells.

Exosomes containing C19MC miRNAs are released from the primary trophoblast and subsequently taken up by recipient cells (Fig. 14.3). This process has also been described for endothelial cells (Delorme-Axford et al. 2013; Cronqvist et al. 2017). These miRNAs are particularly enriched in the trophoblast ("trophomiRs") and stimulate autophagy in recipient cells. Endocytosed viral particles of the endosomal transport pathway are then directed into C19MC-induced autophagosomes. Autolysosomes are formed when these

autophagosomes fuse with lysosomes. This mechanism has previously been described for the degradation of virus-loaded particles (Dumont et al. 2017; Delorme-Axford et al. 2013).

14.7 Circulating Fetal Cells: Their Role in Pregnancy-Associated and Postpartum Diseases

As mentioned in the introduction, the transport of fetal cells in maternal blood was first demonstrated when syncytial knots of the trophoblast were found in the lungs of pregnant women who had died of eclampsia (Lapaire et al. 2007). The finding of increasing numbers of Kleihauer-Betke-positive fetal erythrocytes in the maternal circulation of pregnant women with preeclampsia suggests that this condition is associated with major placental abnormalities. These in turn lead to an increased release of trophoblastic material as well as increased fetomaternal bleeding (Hahn et al. 2005).

Fetal erythroblasts enriched with MACS and examined with FISH for their fetal origin showed a markedly increased number in the blood of women with manifest preeclampsia (Hahn et al. 2005). In addition, it could be demonstrated that this increase can be perceived early in pregnancy, well before the onset of clinical symptoms. This is taken as further evidence that the underlying placental damage is an early event in the course of this syndrome (Hahn et al. 2005).

Our own studies on the release of trophoblastic microparticles demonstrate that these are also released more frequently in preeclampsia. In addition, these microparticles were shown to support the development of neutrophil extracellular traps (NETs) (Hahn et al. 2012b). These traps are found in large numbers in the intervillous space of placentas in preeclampsia, a non-infectious human disease. Because these NETs can promote blood clotting, the overt presence of these traps in the placenta may contribute to the development of placental infarcts and occlusions during preeclampsia (Hahn et al. 2012b).

At the same time, fetomaternal cell transfer can affect maternal health in a completely unexpected way before and after birth, either by ameliorating autoimmune diseases while still pregnant or by increasing their frequency after birth (Nelson 2012).

In the case of rheumatoid arthritis, many pregnant women with the disease experience a reduction in symptoms or even remission during pregnancy (Ostensen et al. 2011). This regulation of disease activity is not solely based on the steroid hormones controlled by pregnancy, such as estrogen and progesterone, but also appears to involve fetal cell transfer (Nelson 2012). In line with this, symptom improvement occurred when there was a mismatch between fetal and maternal MHC molecules. In this way, the transferred fetal cells would downregulate the maternal immune system (Nelson 2012).

On the other hand, certain autoimmune diseases such as scleroderma appear with greater frequency in women after they have given birth (Ostensen et al. 2011). Studies report that diseased tissue from women with scleroderma had traces of fetal genetic material (Nelson et al. 1998). This feature was also evident in other autoimmune diseases such as Hashimoto's thyroiditis, where fetal cells were clearly detected in diseased tissues (Nelson 2012). While long-lived fetal (stem) cells appear to be necessary for these processes, their role in the development of these diseases remains unclear, as their presence in diseased tissues may indicate both tissue repair and initiation of a maternal immune response (Nelson 2012).

14.8 Conclusions

A long-held desire of perinatologists has been to develop techniques that allow non-invasive access to assess fetal genetic status. These techniques would avoid the need for invasive procedures such as amniocentesis, which are associated with significant risk of fetal injury or even fetal loss.

The path to this development meandered from an exploration of circulating fetal cells to cervical trophoblasts to the unexpected detection of placental DNA fragments in maternal plasma.

However, the studies on these different research aspects have significantly contributed to our understanding of many pregnancy-associated processes. In this way, the analysis of circulating fetal cells has shed new light on diseases such as preeclampsia and deepened our understanding of the clinical improvement of autoimmune diseases (rheumatoid arthritis) during pregnancy and the clustered occurrence of such diseases after birth (scleroderma).

In the same way, placental cell-free nucleic acids could be proinflammatory active, while placenta-specific microRNAs could play a key role in preventing viral infections during pregnancy.

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Research Aspects and In Vitro Models

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15.1 Trophoblast Cell Culture Models

Since there is no adequate animal model for the investigation of human placental development, findings on cell biological processes of placental development, such as aspects of trophoblast differentiation, but also immunological and endocrine functions of the human placenta, are to a large extent based on in vitro studies in different cell and tissue culture models of human origin.

15.1.1 Primary Trophoblasts

In general, most protocols for the isolation of villous cytotrophoblasts are based on the Kliman method, which was established in the mid-1980s and initially achieved a purity of the trophoblast population of about 80% (Kliman et al. 1986). In subsequent years, the method was constantly modified, for example by additionally using antibody-coated magnetic beads after enzymatic tissue digestion, thereby further increasing the purity of the isolated trophoblast population (Douglas and King 1989). Based on these approaches, in current protocols villous placental tissue is subjected to stepwise tissue digestion with trypsin, sometimes in combination with collagenase and DNAse, with variations in enzyme concentration and incubation time. The cell fractions resulting from tissue digestion are then separated by centrifugation in a silica gel density gradient (Percoll). In most protocols, this is followed by a final purification step using immunopurification. With further developed protocols based on the Kliman method, different trophoblast subtypes, such as villous and extravillous trophoblasts, can now be obtained from the same placental tissue (Tarrade et al. 2001). If the yield is good, freshly isolated primary trophoblasts can also be frozen and thus used in various experiments and studies. This aspect is especially important for primary trophoblasts of pregnancy pathologies such as gestational diabetes or preeclampsia.

Disadvantages of primary trophoblast culture are the partly limited availability of first trimester placental tissue, interplacental variability, low cell yield, limited cell division activity, and limited transfection efficiency, making gene overexpression or siRNA-mediated gene silencing difficult. In addition, mononuclear or even nonnuclear fragments of the syncytiotrophoblast may occur during trophoblast preparation, affecting analyses of viability and cell fusion rates, which should be considered especially in studies of villous trophoblast differentiation (Huppertz et al. 1999). Fragments of the syncytiotrophoblast can be identified in trophoblast preparations of the term placenta by detection of alkaline phosphatase, as this shows a much stronger expression in the syncytiotrophoblast than in the cytotrophoblast (Frank et al. 2001). However, in the course of cell cultivation and subsequent washing, syncytiotrophoblast fragments can be removed from the trophoblast population, as these, in contrast to cytotrophoblasts, adhere not as good to surfaces of cell culture plates (Guilbert et al. 2002).

15.1.2 Trophoblast Cell Lines

While primary trophoblasts usually have to be freshly isolated for each experiment, and moreover experiments are limited by low cell number, unstable phenotype, poor growth without special culture supplements and contamination with other cell types, trophoblast cell lines represent homogeneous cell populations that ideally can be cultured over numerous passages and exhibit a stable phenotype.

Depending on the tissue status and starting point of cell preparation, trophoblast cell lines can be classified into different groups. On the one hand, trophoblast cell lines have been generated from normal

chorionic tissue as well as malignant choriocarcinomas; on the other hand, embryonic carcinoma cell lines have also been described, which show some potential for trophoblast differentiation. The spectrum of described trophoblast cell lines is enormous and is based on different isolation techniques and different gestational weeks of the starting tissue as well as different methods for the establishment of the cell lines.

The wide spectrum of different trophoblast cell lines also requires a clear phenotypic characterization with detection of trophoblast-specific properties. The most common markers for the detection of a trophoblast phenotype are human chorionic gonadotropin (hCG), human placental lactogen (hPL), and various keratins involved in cytoskeletal assembly as intermediate filaments (King et al. 2000). Although these markers are generally accepted in trophoblast characterization, it should be mentioned here that hCG and hPL are also expressed in some rare tumor cell lines. Therefore, in addition to endocrine activity, the detection of keratin-7 (also termed cytokeratin-7) is often used for phenotyping, since this intermediate filament is exclusively produced by trophoblasts at the uteroplacental unit, with the exception of uterine glandular epithelial cells. Under in vitro culture conditions, trophoblast cell lines partly also express the intermediate filament vimentin specific for mesenchymal cells, which is explained by an epithelial-mesenchymal transition—the transition of epithelial cells into cells with mesenchymal properties. On the other hand, keratin expression seems to be influenced by extracellular matrix proteins, as some trophoblast cell lines maintain keratin-7 expression only on specially coated culture plates, but abandon it in the absence of extracellular matrix proteins (Manyonda et al. 2001).

In general, trophoblast cell lines can be divided into three groups, while bioinformatic analyses sometimes show considerable differences in the gene expression pattern of primary trophoblasts compared to diverse trophoblast cell lines (Bilban et al. 2010).

Choriocarcinoma Cell Lines

Cell lines derived from gestational choriocarcinomas were the first established trophoblast cell lines, which have now proven to be valuable tools in placental research for almost 50 years. Classic choriocarcinoma cell lines include JAR, JEG-3, and BeWo cells, the latter two cell lines having been established by repeated passages in hamster cheeks, sometimes over many years, and having approximately overlapping expression profiles (Bilban et al. 2010; Novakovic et al. 2011). For example, Hertz was the first to isolate choriocarcinoma cells from metastases of the cerebrum around 1960 after autopsy, which were subsequently transplanted into hamster cheeks and serially transferred 304 times in the hamster over a period of 8 years (Hertz 1959). Only after this long period were the cells again removed from the tumors of the hamster cheeks, cultured with decidua tissue, and finally established as the BeWo cell line (Pattillo and Gey 1968). While JAR and JEG-3 cells are suitable for studying adhesion, migration and invasion, BeWo cells proved to be a suitable cell model for elucidating molecular biological processes of villous trophoblast differentiation and fusion (Hannan et al. 2010).

One disadvantage of choriocarcinoma cell lines is that, apparently due to their long existence, different lines developed, some of which exhibit large variances with regard to their differentiation capacity (Orendi et al. 2011). Furthermore, in the case of BeWo cells, for example, the exact gestational age cannot be determined because, as already mentioned, this cell line arose from an advanced metastatic choriocarcinoma. Thus, experimental results obtained with this, but also other choriocarcinoma cell lines, cannot be related to different gestational periods.

Trophoblast Hybridoma Cells

To link phenotypic features of primary trophoblasts with the advantages of choriocarcinoma cell lines, the hybridoma technique was used. In this technique, primary trophoblasts were fused in vitro with the hypoxanthine-guanine phosphoribosyltransferase (HGPRT)-defective JEG-3 chorio carcinoma cell clone AC1–1 using polyethylene glycol (PEG) and subsequently selected in an appropriate culture medium. Accordingly, for example, when generating the hybridoma cell line ACH1P, normal diploid male primary trophoblasts were isolated from a term placenta, placed in culture for 1 day, and then fused with hypertriploid AC1–1 choriocarcinoma cells (Frank et al. 2000). Cytogenetic analysis of the resulting hybridoma cell line, ACH1P, revealed an approximately hypertetraploid chromosome set that exhibited AC1–1-specific chromosomal changes but also normal sex chromosomes of the male primary trophoblast. Interestingly, cell fusion and subsequent initial mitotic divisions of the resulting hybridoma cell lines resulted in the loss of individual chromosomes (Frank et al. 2000).

Another example of a trophoblast hybridoma cell line is ACH-3P, which, in contrast to ACH1P, was generated by fusion of the choriocarcinoma cell line AC1–1 with primary first-trimester trophoblasts (Hiden et al. 2007). Cytogenetic studies of the resulting ACH-3P cells indicated a male cell line that has between 94 and 98 chromosomes. Interestingly, a higher similarity between ACH-3P and primary trophoblasts, than between ACH-3P and AC1–1, was detected by microarray. Since this cell line also exhibits invasive properties and good expression of trophoblast-specific markers, it represents a useful model to study the first trimester of pregnancy.

Transformed Trophoblasts

In contrast to cell lines derived from gestational choriocarcinomas, there are also trophoblast cell lines derived from non-cancerous tissue and a number of different transformation methods. These include, on the one hand, trophoblast cell lines that constitutively express telomerase (trophoblast cell line SWAN-71) and, on the other hand, were transformed with the SV40 large T antigen (cell lines HTR8/SVneo and SGHPL) or by means of retroviral vectors (cell line TEV-1) (Novakovic et al. 2011).

All of these cell lines originate from placental tissue of the first trimester and show an extravillous phenotype. While choriocarcinoma cell lines were cultured for far more than 200 passages, transformed trophoblasts show a rather lower proliferation capacity, which is for instance ten passages for SWAN-71. The trophoblast cell line SGHPL also exhibits a rather limited lifespan of up to 25 passages, but shows no signs of transformation, such as loss of cell contact inhibition, the property of cells to stop cell growth and division above a certain cell density. When cultured on extracellular matrix proteins, such as laminin, gelatin, fibronectin and type IV collagen, these cell lines show keratin-7 expression and prove to be motile and invasive, which is why these cell lines are often used for trophoblast invasion studies (Shiverick et al. 2001).

15.2 Placental Explant Cultures

The cultivation of placental villi in vitro has a long tradition, with the first reports being published as early as the 1940s and 1950s. In this early era, placental pieces were used to analyze oxygen consumption, and later, the transport of amino acids and other small molecules (Miller et al. 2005). In the early 1990s, placental explant culture was further

deepened, with both free-floating placental villi and placental villi attached to extracellular matrix being cultured (Genbacev et al. 1992). In this way, not only the influence of extracellular matrix on trophoblast cell column formation could be studied, but also mechanisms of extravillous trophoblast differentiation and invasion.

In general, placental explant culture can be used to analyze placental functions and viability under different experimental conditions, and also to compare placental tissue from pathological pregnancies with healthy placental tissue of comparable gestational age. Although placental villi are composed of many other cell types besides villous trophoblast, such as mesenchymal connective tissue cells, myofibroblasts, smooth muscle cells, endothelial cells, and macrophages (Hofbauer cells), the main focus of most placental explant studies is on trophoblast functions. The advantage of placental explant culture over primary trophoblast is due to the fact that the villous trophoblast is maintained in its natural tissue environment during placental explant culture. In addition, placental explants are not subjected to intense cellular stress such as primary trophoblasts during the isolation protocol. However, the disadvantage of this model is that culture effects cannot be readily assigned to a single cell type, e.g. villous trophoblast, without using specific detection methods such as in situ hybridization or the more modern and sensitive in situ padlock method (Siwetz et al. 2016).

Due to the fragility of the placental villi, the tissue should be manipulated extremely gently but quickly during the preparation. Since the occurrence of tissue degeneration and detachment of the syncytiotrophoblast in the placental explant culture has been described in isolated cases, a careful microscopic evaluation of hematoxylin-eosin (HE)-stained tissue sections is recommended after the explant culture in order to check tissue integrity, especially of the syncytiotrophoblast. In addition, the integrity and functionality of the villous trophoblast can be evaluated in explant culture using trypan blue, glucose consumption, and measurement of lactate dehydrogenase and alkaline phosphatase release (Miller et al. 2005).

Basically, placental tissue from the first and third trimester of pregnancy is used for the preparation of placental explant cultures. Term placentas are promptly obtained either by natural vaginal delivery or cesarean section in the delivery room and are mostly used to study transport, gene expression and hormone synthesis mechanisms. In contrast, first-trimester placental explants are prepared from villous placental tissue from elective surgical pregnancy terminations or chorionic villus sampling and are used to study early processes of placentation, such as differentiation of villous and extravillous trophoblasts, formation of trophoblast cell columns, or trophoblast invasion and migration (Fig. 15.1). Depending on the gestational age of the placental tissue used, placental explant cultures are cultured under different oxygen conditions. To simulate the oxygen conditions of the early, not yet fully perfused intervillous space in culture, placental explants with a gestational age of up to 10 weeks should be cultured at approximately 3% ambient oxygen (Miller et al. 2005). With transition into the second trimester, endoarterial trophoblastic plugs appear to dissolve in uterine spiral arteries, leading to complete perfusion of the intervillous space by maternal blood and consequent increase in the partial pressure of oxygen. For this reason, placental explants of the late first trimester, but especially of the third trimester, are usually cultured at about 8% ambient oxygen. Optimal culture conditions are ideally achieved by cultivation in so-called hypoxia workbenches, as

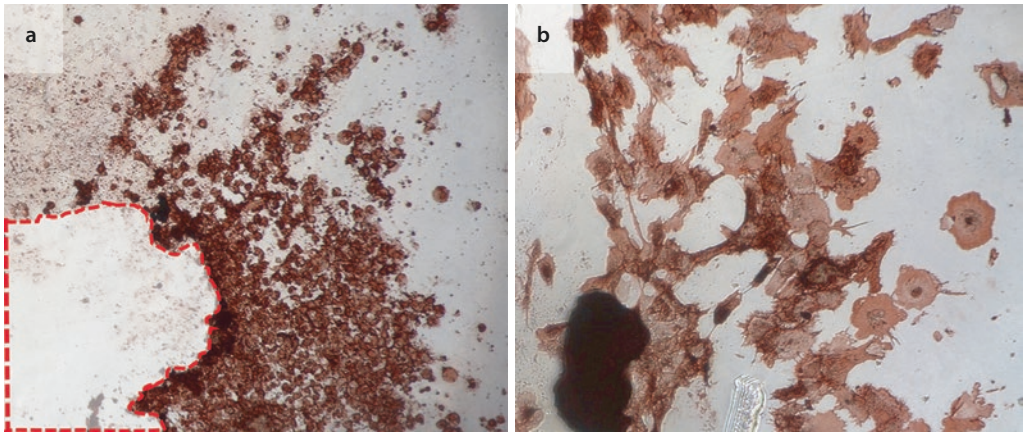

Fig. 15.1 Trophoblast migration. **a**, **b** Cultivation of placental villi on collagen I coated culture plates leads to migration of extravillous trophoblasts, which detach from the villus and migrate as isolated cells. **a** The *red dashed line* indicates the location of the placental villus, which was removed after cultivation. **b** The migrated cells are fixed and the expression of the extravillous trophoblast marker HLA-G is detected by immunohistochemical staining

these allow precise adjustment of the oxygen concentrations and are equipped with airlocks and one or more separate incubators. This allows manipulations of the explant culture, such as medium changes, without fluctuations in the oxygen concentration, which means that placental explants can be cultured over a period of up to 12 days (Miller et al. 2005).

15.3 Endothelial Cells

The endothelium is a single layer of specialized epithelial cells called endothelial cells that line the walls of blood and lymphatic vessels. The placenta is a highly vascularized organ and thus also rich in endothelial cells—in the term placenta, the endothelial surface can reach a size of 15 m^2 (Burton et al. 2009). While other organ-specific blood vessel systems (e.g. lung or heart) are difficult or invasive to access, e.g. for clinical diagnostic biopsies and then only in small quantities, the term placenta is quite easily available after birth and even larger vessels can be used for endothelial cell isolation. Endothelial cells can also be isolated from vessels in the umbilical cord.

15.3.1 Human Umbilical Vein Endothelial Cells (HUVECs)

HUVECs, human umbilical venous endothelial cells, are cells obtained by flushing the umbilical vein with collagenase enzyme solution. The cells were first isolated in the 1970s (Jaffe et al. 1973), and protocols and culture media for HUVECs have since been optimized (Davis et al. 2007). Because the collection is straightforward, and the cells are exceptionally well characterized, HUVECs are also available commercially. Various suppliers sell HUVECs, isolated from donor pools or from single individuals; nevertheless, many research groups also isolate HUVECs on their own. HUVECs express important markers for endothelial cells, such as von Willebrandt factor (vWF), CD31, and receptors for various growth factors and cytokines. They are easy to culture and proliferative, although it should be noted that only early passages (= propagation steps in vitro) should be used for exper-

iments, as the phenotype of primary HUVECs is not stable. Even when comparing the umbilical cord endothelium in vivo with isolated HUVECs, there are differences; for example, HUVECs have been shown to express insulin receptors both freshly isolated and after several passages (Dekker Nitert et al. 2005), whereas histological studies of the umbilical cord have shown that insulin receptors are not present in the endothelium in vivo (Desoye et al. 1994).

HUVECs are adherent cells, they grow either two-dimensionally as a single layer or in three-dimensional cell culture models as spheres or tubes. Such spheres and tubes are particularly suitable for experiments in which blood vessel formation (angiogenesis) is studied (Irvin et al. 2014). HUVECs are not only used in placental research, but over the years have become an important cell culture model for many pathologies (e.g. arteriosclerosis, cardiovascular diseases).

Due to the unstable phenotype and relatively slow proliferation of primary HUVECs, HUVEC cell lines were also generated by fusing primary cells with tumor cells. However, this fusion may cause the endothelial cells to lose their original properties. As an example, the HUVEC line EA.hy926 can be considered. Although this line still carries most of the endothelial-typical markers (vWF, receptors for growth factors, etc.), the cells are much more susceptible to oxidative stress compared to primary HUVECs (Claise et al. 1999) and hardly respond to stimulation with vascular endothelial growth factor (VEGF), since the receptors for it are reduced in EA.hy926 cells (Baranska et al. 2005). The HUVEC line ECV304 has also been described to show different properties compared to primary cells, e.g. abnormal expression of cytokeratin (Hughes 1996). Therefore, when working with HUVECs, primary cells should be the first choice; if this is not possible, one should be aware of the potential differences compared to primary cells.

In the same way as HUVECs are isolated, it is of course also possible to isolate umbilical arterial endothelial cells (Human Umbilical Artery Endothelial Cells, HUAECs); for this purpose, the umbilical arteries must be cannulated and flushed with collagenase solution. Although the extraction would be similarly straightforward, the use of HUAECs is scarce. This is evident when the terms “HUVEC” and “HUAEC” are entered into the Pubmed search engine. While almost 8000 publications show up in which HUVECs were used, there are not even 50 articles in which HUAECs were used as a model. Nevertheless, HAECS are also commercially available from selected suppliers; they have largely the same morphological characteristics as HUVECs and express the endothelial markers vWF and CD31 like HUVECs.

15.3.2 Primary Placental Endothelial Cells

In addition to the possibility of isolating endothelial cells from the umbilical cord, there is also the possibility of preparing arteries and veins from the chorionic plate and obtaining arterial and venous endothelial cells by perfusing the vessels with a collagenase solution (Lang et al. 2008). The disadvantage here is that the cells grow relatively slowly after isolation, so that it takes several weeks before a sufficient quantity of cells is available for larger experiments.

Although HUVECs are easier to isolate, studies have shown that placental vessels differ ultrastructurally from umbilical cord vessels in situ (Nikolov and Schiebler 1981). Moreover, placental microvascular endothelial cells secrete different vasoactive substances (Lang et al. 2003) ompared to the often used HUVECs and also differ morphologically from them (Lang et al. 2001). Especially for those questions in placental research that deal with blood vessel formation, primary placental endothelial cells may

therefore be a more "natural" model than HUVECs. Like HUVECs, human placental arterial endothelial cells (hpAECs) and their venous partners (hpVECs) grow adherently and two-dimensionally. For their growth they require an extracellular matrix, e.g. fibrinogen or gelatine, with which the culture plates are coated. It is also possible to culture the cells three-dimensionally in spheres in a fibrinogen matrix or as tubes in so-called Matrigel. With such 3D angiogenesis assays, blood vessel formation in the placenta can be well studied (Irvin et al. 2014), e.g. by treating the cells with different pro- or anti-angiogenic substances, or by switching off certain genes suspected to be involved in vessel formation by small interfering RNAs (siRNAs).

When working with primary placental endothelial cells, it is important to note that venous and arterial cells have different characteristics. Different characteristics with regard to the expression of certain genes and morphology have also been described for other types of endothelial cells (Chi et al. 2003). These differences are probably due to the microenvironment of the cells, especially with regard to pressure and fluid dynamics in the different vessels.

When cultured in a culture plate without flow, hpAECs have a polygonal appearance, they grow in the classical cobblestone pattern described for endothelial cells (Fig. 15.2a). They express genes that are typical for the arterial phenotype, e.g. Hey-2, Depp and Connexin 40. hpAECs respond to stimulation with VEGF with increased cell proliferation.

hpVECs are spindle-shaped cells that grow in small colonies (Fig. 15.2b). Compared to hpAECs, they have a more juvenile phenotype; they express more genes that are relevant in development, e.g. Gremlin, Mesenchyme Homeobox 2 and DSC54. They hardly respond to stimulation with VEGF; their proliferation is rather regulated by placental growth factor (PlGF). Unlike hpAECs, which have a mature phenotype, hpVECs are able to differentiate into adipocyte- or osteoblast-like cells (Lang et al. 2008). However, this differentiation does not occur spontaneously, but only through appropriate stimulation of the cells.

In general, both hpAECs and hpVECs have stable morphologies and phenotypes that are maintained over multiple passages in cell culture in vitro. However, the lifespan of hpAECs and hpVECs is limited to about 12 passages.

When designing your own experiments, it is important to consider the differences between the cell types and to choose accordingly whether to work with arterial or venous placental endothelial cells.

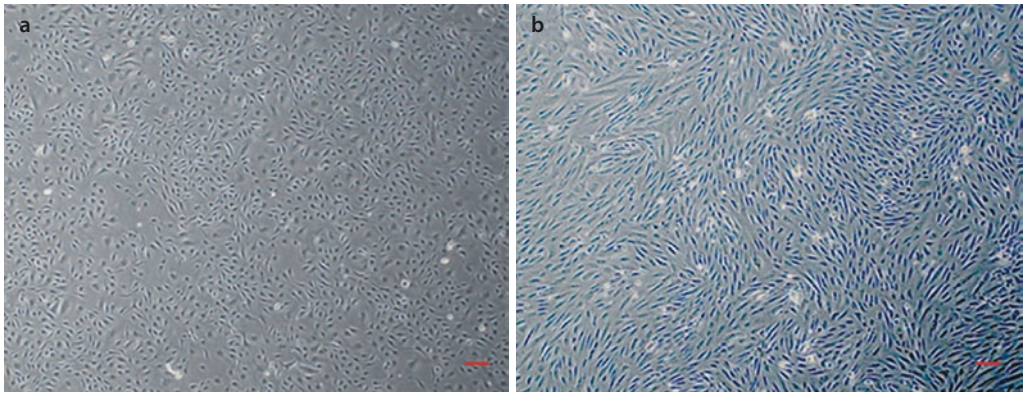

Fig. 15.2 **a**, **b** Primary placental endothelial cells. **a** Placental arterial endothelial cells (hpAECs). **b** Placental venous endothelial cells (hpVECs). The size bars (*red*) correspond to 50 μm

15.4 Placental Macrophages

In addition to methods for the isolation of trophoblasts and endothelial cells, methods for the isolation of macrophages from decidua and placenta have been established and further developed since the 1970s. Apart from their function as phagocytes in inflammatory processes in the placenta, placental macrophages have also been studied with regard to their role in

- vasculogenesis and angiogenesis in the placenta (Seval et al. 2007),
- maternal tolerance towards the fetus (Svensson-Arvelund et al. 2015) and
- the maintenance of pregnancy (Matsubara et al. 2003)

in in vitro studies. In contrast to trophoblasts, macrophages from the decidua and placenta must always be freshly isolated as primary cells, since no immortalized cell lines have been generated to date.

15.4.1 Decidual Macrophages

The decidua arises from the maternal endometrium, so strictly speaking it is not part of the placenta. Nevertheless, decidual cells are well studied, since the decidua can be obtained relatively easily both after elective pregnancy terminations in the first trimester and after birth.

Like the decidua, decidual macrophages are maternal in origin (Bulmer and Johnson 1984). Macrophages have been shown to account for approximately 20% of total decidual cells in the first trimester; this proportion increases to as much as 40% by the third trimester (Loke et al. 1995). Due to their considerable quantity, it is obvious to isolate these cells for in vitro experiments in order to be able to describe their functions.

The first isolation protocols have been in place for nearly 30 years. The method was tested and established with decidua from the first trimester as well as after birth (Vince et al. 1990). Similar to trophoblast isolation, decidual tissue is enzymatically digested in two steps: (1) with protease type XIV and dispase to loosen the tissue without releasing cells, and (2) with a mixture of collagenase, hyaluronidase, and DNase to release the cells from the tissue composite. Subsequently, the different cell populations are separated using a Percoll density gradient and analyzed using Fluorescence Assisted Cell Sorting (FACS). With this isolation method, a purity of 95% with a viability of >90% can be achieved.

A similar method for the isolation of decidual macrophages in the third trimester was also described by Narahara (Narahara et al. 1993) and has been continuously developed since then (Narahara et al. 2003). In this method, the first digestion with protease XIV and dispase is bypassed; instead, an additional enrichment of mononuclear cells in a Ficoll-Hypaque gradient was introduced before the Percoll density gradient. Although viability of >90% is also achieved here and the yield of about 15×10^6 cells/g tissue is remarkably high, this method isolates a rather low amount of pure macrophages, as illustrated by the proportion of CD14-positive cells (<30%) in the quality control by FACS (Narahara et al. 2003).

15.4.2 Hofbauer Cells

Tissue-resident macrophages in the villous structures of the placenta are called Hofbauer cells (HBCs). In contrast to the maternal macrophages of the decidua, these cells are of fetal origin (Wilson et al. 1983; Kawata et al. 1984). The first protocols for isolation of HBC by trypsin digestion of term placental tissue were introduced about 40 years ago (Moskalewski et al. 1975; Wood 1980). Other approaches used collagenase in combination with or without trypsin (Wilson et al. 1983) or included protease type XIV and hyaluronidase (Sutton et al. 1989). In all protocols, digestion is followed

by cell separation via a Ficoll-Hypaque gradient, a Percoll density gradient, or both gradients in succession (Wilson et al. 1983; Uren and Boyle 1985; Sutton et al. 1989). In addition, biological properties of HBCs can be used to further purify the isolated cells. First, macrophages adhere better to the plastic of cell culture plates than most other cells without further treatment and are also very resistant to detachment using trypsin. This can be used to detach all contaminating cells 1–2 h after plating out the cells with excessive washing or enzymatic treatment, while the HBCs remain attached. For example, viability of >90% and purity of >95% can be achieved with such treatments (Wilson et al. 1983). Second, macrophages, in their capacity as immune cells, have numerous surface receptors that can be exploited in purification. One technique for this is "rosetting," in which antibodies to HBC proteins are anchored in the membrane of erythrocytes, which are then incubated with an HBC cell suspension. HBCs bind to the red cells, while other cells remain unbound and can be separated by centrifugation. The erythrocytes then still need to be lysed to obtain pure HBCs (Sutton et al. 1989). Another common option is purification by magnetic beads to which an antibody to the macrophage marker CD68 has been coupled (Cervar et al. 1999). Both rosetting and purification via CD68 involve "positive immunoselection," i.e. HBCs are bound via an antigen and all other unbound cells are removed by washing.

Since positive immunoselection may result in undesirable proinflammatory activation of macrophages, which could affect results especially in functional assays, recent protocols for isolation of HBCs use negative immunoselection, i.e. all non-HBC cells are bound via antibodies on magnetic beads and removed by a magnet, while HBCs remain in suspension. For negative selection, e.g. antibodies against Epidermal Growth Factor Receptor (EGFR) are used on cytotrophoblasts, which are then removed (Wetzka et al. 1997), leaving HBC, which then have a purity of >90% (measured on $CD68^+$ cells). In a second step, fibroblasts can also be removed using an antibody against CD10 (Tang et al. 2011). With this method, the purity increases to almost 99% (measured on $CD163^+$ cells) with a yield of approx. 1.5×10^6 cells/g tissue.

There is also the possibility of isolating Hofbauer cells from first trimester placental tissue, the protocol for this is very similar to the methods used for isolation from term tissue: digestion is unnecessary, the tissue is very finely minced and applied sequentially to Ficoll Hypaque and Percoll gradients, then the cells are enriched according to their surface molecules by rosetting (Zaccheo et al. 1989). However, the starting material is the limiting factor; 10–15 tissue samples must be collected and pooled to perform an experiment (Zaccheo et al. 1989).

Immediately after isolation, Hofbauer cells look mostly round and, characteristic for these cells, show a large number of vacuoles (Fig. 15.3a). After about 2 days in culture, the cells begin to elongate and form projections (Fig. 15.3b). They are capable of phagocytosis and, for example, of taking up cholesterol (Fig. 15.3c). In addition to the relatively laborious isolation, a disadvantage is that the cells can only be kept in culture for 7–10 days, and there is no possibility of freezing the cells and using them at a later time point. In addition, HBCs are non-proliferative, so the yield at the end of isolation is a limiting factor in the design of further experiments. However, as human primary cells, HBCs have high plasticity and thus can be used in a variety of assays to investigate their role in placental angiogenesis (Loegl et al. 2016), vertical transmission of infectious diseases (Johnson and Chakraborty 2012; Simoni et al. 2017), or inflammatory diseases of the placenta (Ben Amara et al. 2013; Kim et al. 2008).

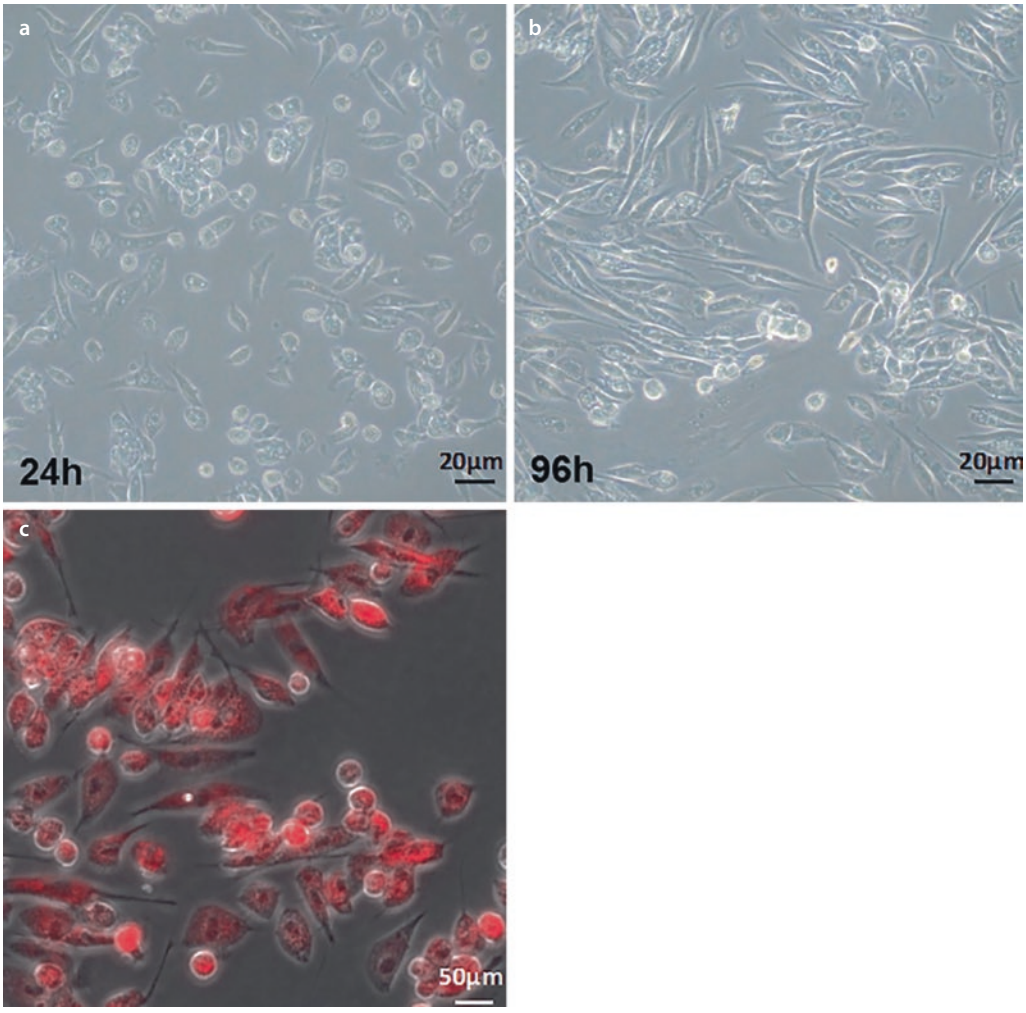

Fig. 15.3 **a**–**c** Hofbauer cells (HBCs) in culture. **a** 24 h after isolation HBCs are still small and round. **b** 96 h after isolation HBCs are already elongated and form colonies. **c** HBCs after phagocytosis of fluorescence-labeled LDL cholesterol. (With kind permission of Jelena Lögl)

15.5 Placenta Ex Vivo Perfusion

The human placenta is delivered immediately after delivery of the child and is thus promptly available for ex vivo examinations of the tissue. Ideally, the structural integrity of the tissue is not affected by the birth process, and both the fetal vasculature of the placenta and the intervillous space filled with maternal blood remain intact. The structural integrity of the tissue, as well as the fact that metabolic functions of the placenta are still maintained after delivery, provides the basis for studies that can be performed without risk to mother and newborn. In a large number of placental perfusion studies over the past decades, both nutrient and gas exchange between mother and child have been investigated, but also the transport or transfer of antibodies, hormones, growth factors, active substances, nanomaterials or environmental toxins. Placental perfusion also offers the possibility to investigate the function of the pla-

centa in different pregnancy pathologies, such as preeclampsia, intrauterine growth restriction or gestational diabetes, and to compare them with uncomplicated pregnancies.

15.5.1 Methodology

As mentioned above, the maintenance of placental cell barriers is crucial for the function of the placenta and thus represents a particular challenge for the successful performance of a perfusion experiment. Today, a widely used method is the perfusion of one functional unit of the placenta, a so-called cotyledon. This method is founded on work by Panigel, Schneider and Dancis (Panigel 1962; Schneider et al. 1972) and has been continuously developed. In addition to tissue integrity, an important factor is the shortest possible time interval between placental delivery and cannulation of the corresponding fetal vascular pair (artery and vein) in order to keep the ischemic period as short as possible. Initially, fetal blood is flushed out via the cannulated artery and exits the placenta via the cannulated fetal vein. Subsequently, the selected cotyledon can be fixed in a temperature-controlled perfusion chamber, connected to a tube and pump system and supplied with culture medium. To ensure the most physiological conditions possible, the culture medium is heated to 37°C and the oxygen content is reduced in the fetal circulation using a gas mixture of N_2 and CO_2. The integrity of the fetal vasculature is checked at this time by the volume of circulating culture medium. An intact vascular system returns >95% of the medium used to the vein exiting the placenta. The next step is to establish maternal circulation, this is done by passing blunt cannulas through the basal plate into the intervillous space and pumping the culture medium through it. The culture medium leaves the intervillous space by diffusion and can be returned to the maternal reservoir. After a preperfusion period of about 30 min, the planned experiment can be performed. Crucial for the success and validity of an ex vivo perfusion experiment are, on the one hand, the guarantee of conditions that are as physiological as possible and, on the other hand, the constant monitoring of parameters that reflect the integrity and vitality of the tissue.

The culture medium used should correspond as closely as possible to the composition of maternal and fetal blood. For this purpose, a bicarbonate-buffered basic medium with glucose, amino acids, vitamins and minerals is often mixed with serum albumin (of bovine or human origin) or dextran as a plasma expander. Dissolved gases and thus the degree of oxygenation of the culture medium represent another important class of substances. In blood, hemoglobin is the most important O_2 and CO_2 transport system. In the placental intervillous space oxygen partial pressures of about 100 mmHg are reached, these are much lower in the umbilical cord blood with 20–30 mmHg. In the perfusion experiment, the culture medium is gassed with a gas mixture of N_2 and 5% CO_2 in order to approximate the oxygen partial pressure in the fetal medium to physiological values on the one hand and to keep the pH of the bicarbonate buffer system stable at pH 7.4 on the other. The flow rates in the experimental maternal and fetal circulation are adjusted to the average size of a perfused cotyledon to keep the back pressure of the fetal vasculature low and to maintain the integrity of the endothelium. The intervillous space does not have a vascular system, but maternal blood circulates around the fetal villous tissue. To maintain a pressure gradient from the maternal to the fetal circulation, maternal flow rates must be selected approximately three times higher than fetal flow rates.

Fetal vascular backpressure is continuously monitored by micropressure catheters or similar sensors. During the establishment phase of the perfusion experiment, fetal placental vascular back pressure continuously decreases until a stable value of <50 mmHg

is reached. Excessive vascular back pressure may be caused by coagulation of residual blood and further leads to endothelial damage and consequent inadequate tissue perfusion. The sensitive and critical parameters, such as pH and efficient oxygen transport from the maternal to the fetal circulation, are checked at regular intervals during the perfusion experiment using sensors or a blood gas analyzer. In addition to efficient oxygen and carbon dioxide exchange between maternal and fetal circulation, passive diffusion of lipophilic substances such as antipyrin provides a good measure of the overlap between the two circulations and thus of the general effectiveness of substance exchange in each experiment. An indication of the metabolic activity of the tissue is provided by glucose consumption and lactate formation during the experiments. The verification of the above parameters and a possible adaptation of the experimental conditions guarantee the comparability of experiments and the best possible replication of the in vivo situation (Fig. 15.4) (Mathiesen et al. 2010).

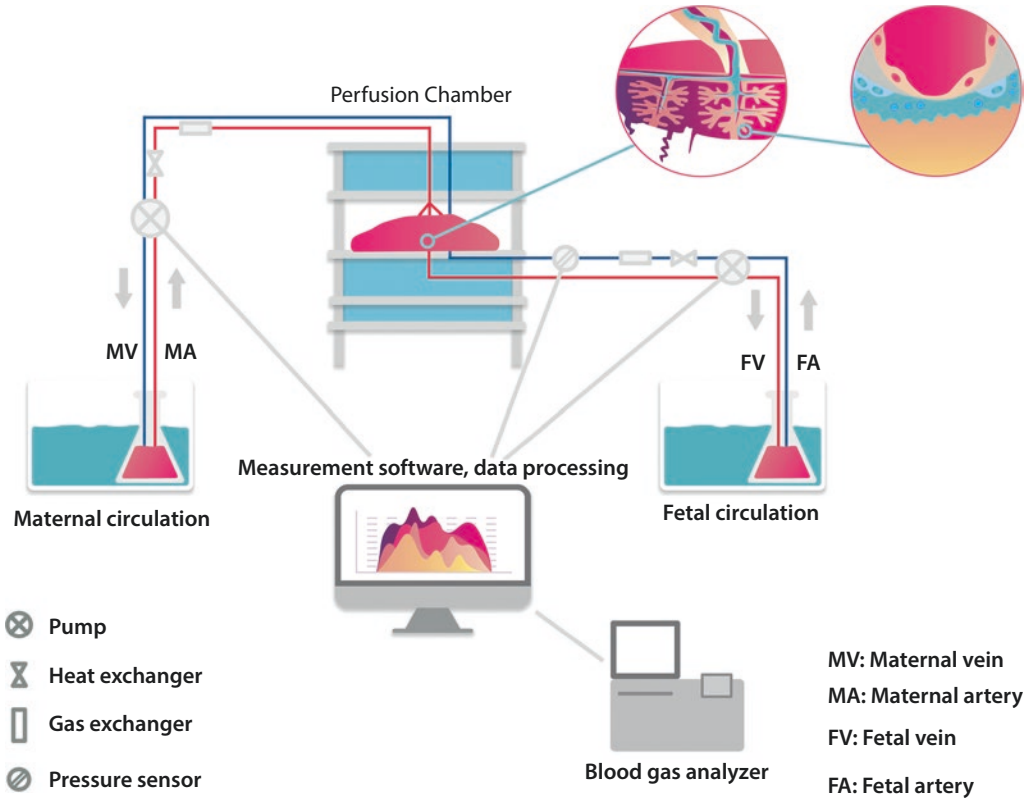

Fig. 15.4 Set-up of a placenta perfusion experiment. The cannulated placenta is placed in the prewarmed perfusion chamber and supplied with nutrient medium via the maternal and fetal circulation separately. Pump, heat exchanger and gas exchanger ensure adequate temperature control (37°C) and optimal O_2 content and pH value in the nutrient medium. Metabolic parameters such as glucose consumption, lactate production, pH-value, O_2 and CO_2 saturation are determined during the experiment in the maternal and fetal nutrient medium by means of a blood gas analyzer and documented collectively. They provide information about the vitality of the placental tissue and thus the quality of the experiment. *MA* maternal artery, *MV* maternal vein, *FA* fetal artery, FV fetal vein. (With kind permission © PlaZentaTox Labor, Assoz. Prof. Wadsack)

15.5.2 Applications in Research

In the early years of ex vivo perfusion, the focus of research was mainly on the elucidation of transport tasks and physiological processes in the placenta. Of particular interest were substrates that play a crucial role in fetal development, such as placental consumption and transport of oxygen and glucose (Challier et al. 1976; Hauguel et al. 1983), as well as the transport of fatty acids and amino acids (Dancis et al. 1973; Schneider et al. 1979). These insights have recently been extended by combining experimental data with mathematical models (Perazzolo et al. 2016; Lewis et al. 2013). The use of mathematical models to predict transport behavior of nutrients, but also of active compounds, can provide important information for future developments.

Today, an important aspect of placenta perfusion studies is the fact that a large number of women require medication for the treatment of diseases in the course of pregnancy. Since there are reliable data on the effects on the fetus for only a few therapeutic agents, it is often up to the physician to balance the benefits and risks of treatment for the woman and the unborn child. In order to test the safety of active substances with regard to their placental compatibility, cell culture models are only suitable to a limited extent, since only the uptake into an isolated cell type can be determined. Furthermore, the structure of several cell layers and the circulating blood in two independent circuits are not taken into account. When using animal models, the direct translation of the results to the human organism is always a factor of uncertainty due to differences in tissue structure as well as in transport physiology between species. Ex vivo perfusion offers the possibility to determine the transfer of drugs across the multicellular barrier of the human term placenta under "physiological blood flow." Furthermore, pharmacokinetic factors describing drug transport, binding and metabolism can also be determined by ex vivo perfusion experiments. Ex vivo perfusion is useful for studying drugs already on the market, as well as for clinical testing during the development of new drugs used to target the mother or unborn child (Hutson et al. 2011). Nanomaterials can make a further contribution to future targeted medication of the mother, unborn child or placenta. The physicochemical properties that, for example, nanoparticles with and without an active substance must exhibit in order to cross the placental barrier or remain in the maternal circulation can be elucidated by means of ex vivo placental perfusion without risk to mother and fetus (Buerki-Thurnherr et al. 2012).

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Maternal Disease Affecting the Placenta: Diabetes Mellitus

Tanja Groten

Contents

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16.1 Definition and Epidemiology

16.1.1 Definition

Diabetes in pregnancy is present when a diabetic woman becomes pregnant or when glucose intolerance develops during the course of pregnancy. A glucose tolerance disorder diagnosed for the first time during pregnancy by means of an oral glucose tolerance test (oGTT) can be either a previously unknown pre-existing diabetes mellitus or gestational diabetes.

In the case of pre-existing diabetes, the affected pregnant women display very different risk profiles. In the case of type 1 diabetes mellitus, there is an insulin deficiency, which is absolute in most cases. The patients are dependent on the administration of insulin. Often the disease has existed since childhood. With a long course of the disease, secondary complications are often already present, predominantly caused by hyperglycemia-related endothelial damage with consecutive vasculopathy. As a consequence, hypertension, renal damage and retinopathy may develop. In the case of type 2 diabetes, there is insulin resistance, often requiring very high amounts of insulin supplementation. The patients are often overweight and may suffer from the sequelae of metabolic syndrome.

The physiological onset of insulin resistance in the second half of pregnancy leads to hyperglycemia in pregnancy in women with previously compensated insulin resistance or a latent insulin secretion defect. The patient develops gestational diabetes (GDM), a glucose tolerance disorder in pregnancy.

Due to these typical metabolic changes and the presence of obesity in the majority of affected pregnant women, "classic" GDM is considered a form of pre-type 2 diabetes, which is characterized by significant insulin resistance and impaired beta cell function (Kautzky-Willer 2005). Approximately 50% of patients develop type 2 diabetes after GDM.

16.1.2 Epidemiology

The data on the prevalence of gestational diabetes vary in the different epidemiological studies depending on the population group studied, the timing of the examination and the screening and diagnostic approach used (implementation of general or risk-adapted screening, inconsistent diagnostic criteria). The more frequently GDM is searched for in pregnant women, the more frequently diabetes risks occur in the population group examined and the lower the diagnostic thresholds of the test procedure used, the more "frequently" GDM is diagnosed.

Accordingly, in recent literature worldwide, the prevalences of GDM vary from 1.9% to 25% (Guariguata et al. 2014; Ignell et al. 2014; Mwanri et al. 2015).

In 2015, around 715,000 births were recorded in Germany by the Institute for Quality and Transparency in Health Care (IQTIG). The evaluated data represent 99.6% of the expected births. Among the mothers, GDM was present in 35,399 cases (4.95%) (Fig. 16.1). In 2017, Melchior et al. published in the German Medical Journal a survey of the diagnosis of GDM from health insurance claims data in the outpatient sector and found a prevalence of GDM of 13.2% in 567,191 recorded pregnancies. (Melchior et al. 2017). Common to all epidemiological studies is the increasing incidence over the last 10 years, which has continued even after the diagnostic cut-off values have been largely standardized (Fig. 16.1). According to IQTIG and AQUA (Institute for Applied Quality Promotion and Research in Health Care) surveys, the proportion of pregnant women with pre-existing diabetes mellitus remains constant at around 1%. Here, too, an increase in numbers is found, which is mainly due to an increase in pregnant women with pre-existing type 2 DM.

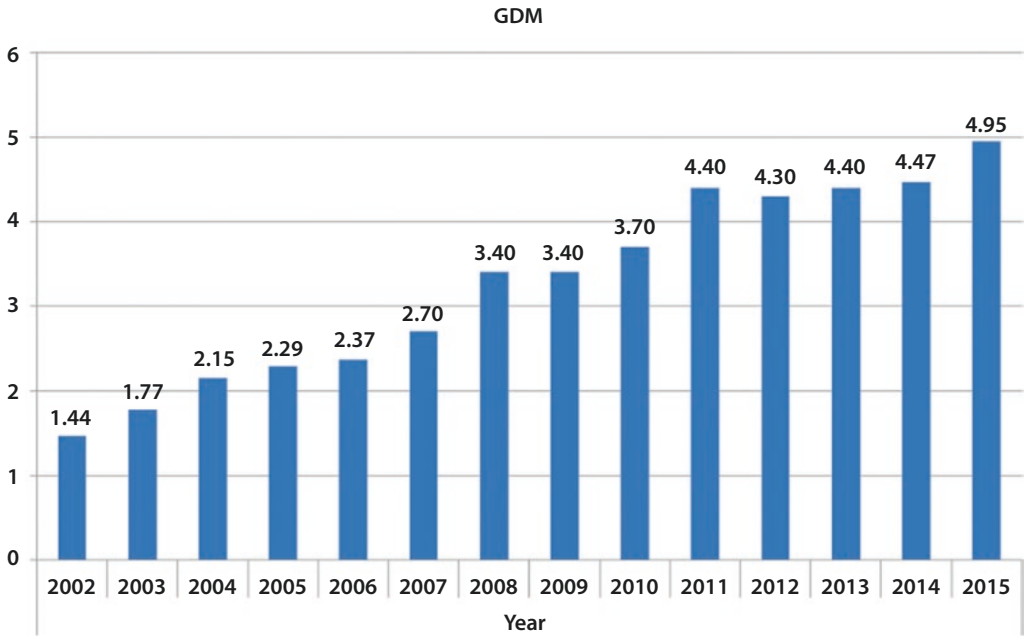

Fig. 16.1 Relative frequencies of gestational diabetes (GDM) in Germany 2002–2015 according to the Institute for Quality and Transparency in Health Care (IQTIG)

16.2 Effects of Hyperglycemia in Pregnancy

The transfer of glucose across the placenta follows the concentration gradient of the glucose level between mother and child. Consequently, maternal hyperglycemia is followed by fetal hyperglycemia. While the mother has an insulin deficiency and cannot adequately metabolize glucose, the child produces increased insulin as a result of the glucose oversupply. Hyperinsulinemia develops in the fetus. The expression of glucose transporters and other structural changes in the placenta do not alter this basic principle; especially in the third trimester, the transfer of glucose from mother to child depends solely on the difference in concentration between the maternal and fetal compartments (Weiss 2002; Desoye and van Poppel 2015).

The increased intrauterine glucose supply leads to increased fetal insulin secretion, deposition of glycogen in the fetal heart muscle and formation of white adipose tissue. As a result of increased intrauterine erythropoietin levels, fetal hematocrit increases. In the presence of hyperinsulinemia, fetal surfactant formation is reduced. At birth, diabetic fetopathy with hypoglycemia, respiratory disorders, polyglobulia, hypocalcemia, hypomagnesemia, and hyperbilirubinemia is seen in varying degrees. In severe cases, intrauterine fetal death may occur.

Intrauterine glucose oversupply also leads to beta-cell hyperplasia in the fetus, which increases the risk of developing obesity and diabetes in children throughout life via multiple mechanisms of fetal programming (Gluckman et al. 2008a, b; Clausen et al. 2008; Schaefer-Graf et al. 2005).

The aim of the treatment of diabetic women during pregnancy is therefore the prevention of maternal hyperglycemia and thus of fetal hyperglycemia and its consequences.

The consequences of maternal hyperglycemia in the child are the effects of fetal hyperinsulinism. If maternal hyperglycemia is avoided, the consequences in the child can be completely avoided.

16.3 Diagnosis and Therapy

16.3.1 Screening and Diagnosis of Gestational Diabetes

An evaluation for the presence of risk factors for diabetes should be performed during the initial presentation of the pregnant woman. If risk factors are present, a diagnostic 75-g oGTT should be performed as soon as the pregnancy is established. The German maternity guideline prescribes a 50-g screening test for all pregnant women at 24–28 weeks of gestation. Abnormal results must then be confirmed by diagnostic 75-g oGTT (Fig. 16.2) (AWMF 2011). The cut-off values of the 75-g-oGTT recommended by the International Association of Diabetes and Pregnancy Study Groups (IADPSG) are shown in the following overview.

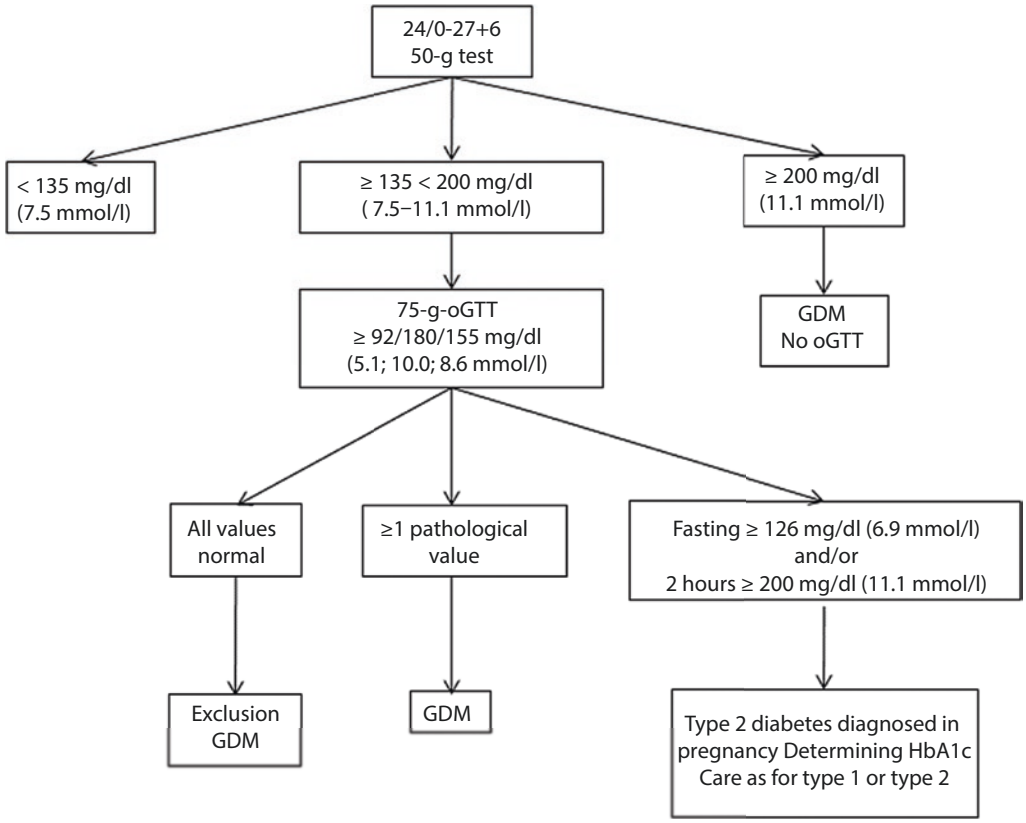

Fig. 16.2 Diagnostic procedure to exclude gestational diabetes (GDM) in pregnancy. (According to the revised version of the German "AWMF-Leitlinie Gestationsdiabetes 2011", not yet published)

Threshold Values of the Oral Glucose Tolerance Test with 75 g Glucose (75-g-oGTT) (IADPSG Criteria)

- Fasting glucose: ≥5.1 mmol/l (≥92 mg/dl)
- Glucose after 1 h: ≥10.0 mmol/l (≥180 mg/dl)
- Glucose after 2 h: ≥8.5 mmol/l (≥153 mg/dl)

16.3.2 Therapy

The therapy of diabetes in pregnancy aims at maintaining maternal normoglycemia. The available studies on the relationship between maternal blood glucose levels and the fetal consequences of diabetes show a linear relationship and do not allow cut-off values to be defined (Hyperglycemia and Adverse Pregnancy Outcome [HAPO] Study Cooperative Research Group et al. 2008). Therefore, the setting targets are not yet uniform in the various international guidelines. For Germany, the guidelines for the treatment of gestational diabetes and for the treatment of diabetics in pregnancy recommend a target range that should be interpreted more narrowly or more broadly depending on fetal growth (Table 16.1) (AWMF 2011; 2014).

In order to achieve these adjustment goals, insulin therapy is continuously and closely monitored during pregnancy in the case of pre-existing DM. Existing oral medication for type 2 DM must be switched to insulin as soon as pregnancy is established and ideally even as soon as pregnancy is considered.

In the case of GDM, treatment consists of a multi-pillar concept of medical education, self-monitoring of blood glucose, moderate dietary changes, exercise therapy and, if necessary, drug therapy. The education about the recommendations for weight gain during pregnancy as a function of maternal baseline BMI is of particular importance (Table 16.2). Controlling weight gain during pregnancy leads to an improvement in insulin sensitivity and improves the maternal and fetal outcome of pregnancy (Rasmussen et al. 2009).

Insulin therapy should only be initiated in GDM if the individual target ranges cannot be achieved with diet and exercise therapy. Short-acting insulins are used as boluses with meals when postprandial target values are exceeded, as well as long-acting insulins for nocturnal insulin resistance with elevated fasting blood glucose levels.

Table 16.1 Target values for blood glucose control in pregnancy

Time	Plasma equivalent	
	mg/dl	mmol/l
Fasting	65–95	3.6–5.3
1 h postprandial	<140	<7.8
2 h postprandial	<120	<6.7

Table 16.2 Recommended weight gain during pregnancy according to the current recommendations of the Institute of Medicine (IOM) of the US National Academy of Sciences

Preconceptional BMI (kg/m²)[a]	Total weight gain during pregnancy (kg)	Weight gain/week in the second and third trimester (kg)
<18.5	12.5–18	0.5–0.6
18.5–24.9	11.5–16	0.4–0.5
25.0–29.9	7–11.5	0.2–0.3
≥30	5–9	0.2–0.3

[a] According to WHO definition

16.3.3 Timing of Delivery in Patients with Diabetes

Diabetic pregnant women are at risk. Delivery should take place in a clinic with an affiliated pediatric clinic in order to ensure optimal primary care for the child. Delivery in a perinatal center is also recommended for insulin-dependent GDM (AWMF 2011, 2014).

Once the calculated date of delivery has been reached and the pregnancy continues, the question arises in every pregnancy as to the further procedure. An indication for termination of pregnancy, i.e. for induction of labor, arises in the event of a situation endangering the child or the mother.

Maternal risk situations arise more frequently in diabetic women than in healthy women in the course of the development of late onset preeclampsia or in the case of progressive deterioration of organ functions on the basis of pre-existing organ damage, e.g. in the case of nephropathy in patients with DM type 1.

Child endangerment situations arise near term from a decline in placental function at the end of pregnancy. Epidemiological studies have shown a constantly increased risk of late placental insufficiency with potentially fatal consequences for the child, seemingly unaffected over the last decades (Smulian et al. 2002). As early as 1992, a large epidemiological study from the USA showed that the risk of peripartum asphyxia and perinatal death decreased steadily over the 20 years from 1961–1988 under the influence of improved medical care for pregnant women and parturients, while at the same time the risk of perinatal complications in insulin-dependent diabetic women remained unchanged over the same period (Fretts et al. 1992). Even the shift of the time of delivery further towards term over the past 10 years has not led to an increase in term stillbirths in the overall collective. In contrast, in the diabetic subpopulation, the risk of stillbirth increased by 25% over the same period (Little et al. 2015). Thus, it appears that there is a risk of failure of placental function near term in diabetic women that has not been captured by current methods of antenatal care and monitoring. For this reason, the recommendation to date is to terminate the pregnancy no later than when the expected delivery date is reached. This also applies to insulin-dependent gestational diabetics.

Histomorphological changes in the placentas of pregnant women with diabetes provide evidence for a placental structural cause that may explain this empirical association.

In the case of dietary normoglycemic GDM and normal fetal growth parameters, if the expected delivery date is exceeded, the procedure is the same as for healthy pregnant women.

Key Points

- Diabetic women who require insulin have a significantly increased risk of sudden placental insufficiency and intrauterine fetal death near term compared with healthy women.
- Patients with DM requiring insulin should therefore be closely monitored until the end of pregnancy and induction of labor when the expected delivery date is reached.
- It is possible that the structural changes in the placenta described in diabetic women are responsible for this increased risk.

16.4 Significance of the Placenta for Glucose Metabolism in Pregnancy

The endocrinological adaptation mechanisms that control the changing food supply to the fetus during pregnancy have not yet been conclusively elucidated and understood. As an endocrine organ, the placenta secretes various hormones during pregnancy

that intervene in the metabolism of the pregnant woman. The secretion of growth hormones from the placenta and also the pregnancy-related altered production of growth hormones in the pituitary gland regulate the supply of nutrients and growth signals to the fetus. Disturbances in placental function and pre-existing endocrinological disorders in the mother influence the balance of the nutrient supply and can cause an undersupply or oversupply of the fetus.

In early pregnancy, placentally produced estrogen and progesterone cause an increase in appetite and thus an increased food intake by the mother. The increased insulin secretion (increase of 60%) then leads to increased lipogenesis in the case of normal insulin sensitivity (Newbern and Freemark 2011). The expectant mother builds up depots for pregnancy. As a result, maternal leptin levels increase. At the same time, under the influence of estrogen, there is an increase in uterine blood flow and prolactin levels. It is likely that estrogen is also important for placental angiogenesis in early pregnancy (Das et al. 2009).

In the late second and third trimester, the mother develops increasing insulin resistance under the influence of placentally secreted hormones. Insulin sensitivity is reduced by 45–70% (Freemark 2006). At the same time, maternal adiponectin levels fall and the leptin/adiponectin ratio increases. After meals, when insulin secretion is high, adipogenesis, gluconeogenesis and energy storage occur. In the fasting state when insulin levels are low, triglycerides and glycogen stores are mobilized, and because of existing insulin resistance, the mother can utilize free fatty acids for energy and saves glucose, amino acids, essential fatty acids, and ketones, which are then available for placental and fetal growth.

The increasing insulin resistance of the mother can be explained in part by the mother's increased progesterone and cortisol levels, which reduce the effect of insulin on skeletal muscle. In addition, secretion of the placenta-specific growth hormone GH-V occurs in the placenta. GH-V secretion is dependent on placental size and maternal glucose levels. High glucose levels inhibit the secretion of GH-V, whereas low levels have a mild stimulatory effect. GH-V acts as an insulin antagonist and decreases insulin action (Newbern and Freemark 2011). As a potent insulin antagonist, GH-V stimulates lipolysis and causes mobilization of maternal fat and glucose depots during fasting periods and low glucose levels. Thus, GH-V has a strong diabetogenic effect. In healthy women, there is a compensatory increase in insulin production, which doubles by the third trimester (Newbern and Freemark 2011). It is assumed that the necessary increase in beta cell mass and capacity is regulated by the lactogenic hormones, human placental lactogen (hPL) and prolactin (Fleenor et al. 2000; Newbern and Freemark 2011).

Under the influence of placental lactogen and rising prolactin from the maternal pituitary, the expectant mother develops central leptin resistance, which, with continued hyperphagia under the influence of GH-V, leads to a steadily increasing flow of nutrients to the fetus.

Key Points

- To ensure a consistent supply of nutrients to the fetus, maternal metabolism undergoes significant changes during pregnancy.
- A phase of increased insulin sensitivity at the beginning of pregnancy is followed, under the influence of placental growth hormones, by the development of insulin resistance with an increase in insulin requirements.
- Compensatory placental lactogen and prolactin cause beta-cell proliferation with increased secretory capacity for insulin.
- Depending on the size and function of the placenta, the extent of insulin resistance in pregnancy can be strong or weak.

16.5 Placental Changes in Patients with Diabetes

In pregnancies complicated by maternal diabetes, a variety of functional and structural changes are found in the placenta. These include, in addition to the usually markedly increased placental weight, various structural changes, often involving maturation defects of the placental villi, increased angiogenesis and the occurrence of necrosis.

In a systematic review in 2015, Huynh et al. summarized the state of knowledge on histomorphological changes in the placenta in diabetes in the literature from 1969–2014 (Huynh et al. 2015a). However, particularly because of the lack of a uniform system for histopathological assessment of placentas, such a review cannot include a summarizing quantitative analysis. Another problem is the inhomogeneity of the studied collectives, with incomplete information on the type of diabetes mellitus, duration of pre-existing conditions, and quality of glycemic control periconceptually and during pregnancy (Huynh et al. 2015a). The results of the individual studies therefore differ with regard to both the pattern of histopathological changes typical in diabetes and their frequencies. In addition, the influence of glycemic control on histopathological changes in the placenta also appears to be important. There are studies that found normal morphology in placentas of well metabolically controlled patients (Mayhew and Jairam 2000). Given the widespread improvements in the care of pregnant women with diabetes over the last 10 years, the histomorphological changes described above may not be found in well-controlled patients today.

Sufficient formation and maturation of vessels in the placenta is the prerequisite for adequate supply of the fetus throughout the course of pregnancy. Pro- and anti-angiogenic factors regulate vasculogenesis and angiogenesis in the placenta. Vasculogenesis in the palcenta begins 21 days after conception with the differentiation of endothelial cells from pluripotent mesenchymal progenitor cells. Subsequently, connection of the resulting vessels occurs, and angiogenesis begins. The vessels begin to grow and branch, a process that continues continuously until the end of pregnancy (Cvitic et al. 2014).

Morphological changes of the vessels in the placenta are frequently described in diabetes. Huynh et al. report two studies demonstrating redundant capillary connections in patients with diabetes (GDM and type 1) (Jirkovská et al. 2002, 2012) and increased placental angiogenesis measured as capillary volume, surface area and length (Mayhew 2002). It is interesting to note that Mayhew explicitly describes an increase in capillary length and volume, but no change in the number of vessels, so it is most likely increased angiogenesis, but not increased vasculogenesis.

Depending on the type of diabetes, the diabetes-specific metabolic changes affect the vascular development at different stages. In the case of pre-existing diabetes, hyperglycemia, acidosis or even hypoxemia (on the basis of maternal vasculitis) can affect placental development already at the stage of vasculogenesis. Persistent hyperglycemia in these patients leads to a permanent influence on placental function and development. In contrast, in gestational diabetes, hyperglycemia occurs for the first time during the second half of pregnancy when vasculogenesis is completed, affecting the process of angiogenesis only then (Cvitic et al. 2014). A disturbance already at the time of vasculogenesis may have different effects than a change only at the stage of angiogenesis (Fig. 16.3).

In the third trimester, the structural changes in type 1 diabetes and GDM are similar despite the different duration of hyperglycemic stress. In type 1 diabetes, there is an increased growth in length as well as an increased branching of the villous capillaries. Similar changes can also be found in GDM (Jirkovská et al. 2002, 2012).

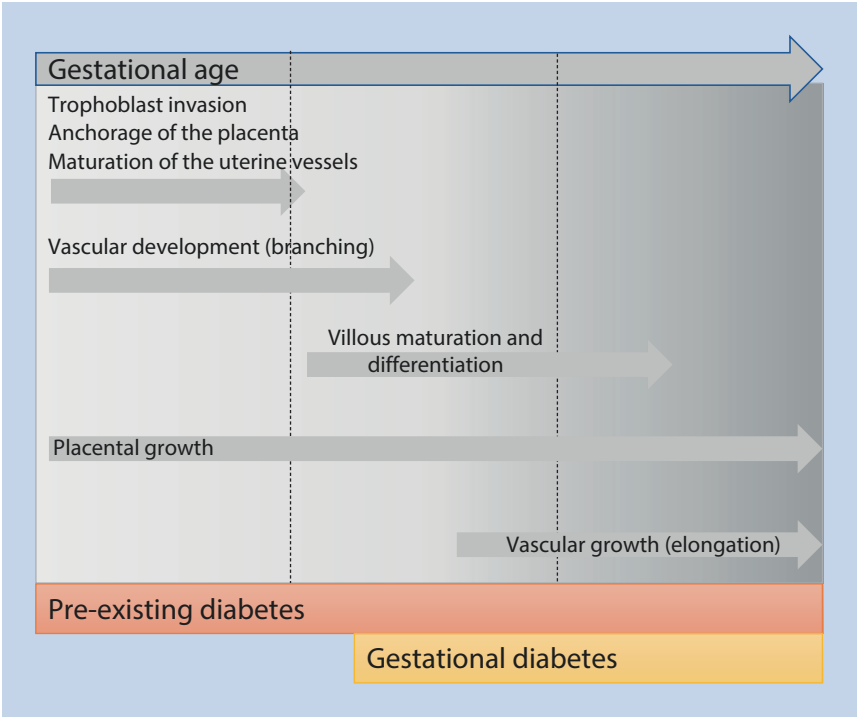

Fig. 16.3 Graphical representation of the phases of placental vasculogenesis and angiogenesis during placental development. The metabolic changes in pre-existing diabetes mellitus or gestational diabetes have different effects because of the time window in which they act. (Modified after Cvitic et al. 2014)

Bernischke et al. (2006) described the cause of the significantly increased placental weight in poorly controlled diabetic women as a consequence of hypervolemia. Histomorphologically, more widely branched terminal villi are found in the placenta as a result of increased angiogenesis, while a disturbance in vasculogenesis leads to a reduced vessel density in less branched villi (► Chap. 1).

16.5.1 Placental Changes in Pre-Existing Diabetes

For pre-existing diabetes, a maturation disorder of the villi and an enlargement of the parenchymatous part of the placenta are the most frequently cited changes. Accordingly, the parenchymal portions of the placenta include the intervillous space, trophoblast layers, and fetal vessels (Huynh et al. 2015b). In this context, data available up to 2014 partly indicate a doubling of the proportion of immature villi at term, which is 14% in the healthy obstetric population (Higgins et al. 2012). However, a reliable statement on the influence of diabetes on villous maturation is not possible, as the authors use widely differing criteria for assessing villous maturity (Huynh et al. 2015b).

Pregnancies of patients with pre-existing diabetes are often complicated by placenta-associated complications such as fetal growth restriction (FGR) and preeclampsia. The morphological changes typical of these are then also found in the placentas of these patients. The risk for the development of FGR and preeclampsia is increased especially in patients with a long duration of the

disease and subsequently existing vasculopathy. The associated restricted perfusion of the placenta has an influence on placental morphology and function at the stage of early placental development, irrespective of the existing metabolic disorder. Placentas with a lower weight often develop. It is possible that this developmental disorder of the placenta in turn interferes with the endocrinological rearrangement of maternal metabolism. Lower production of GH-V and later of hPL may affect maternal glucose homeostasis during pregnancy.

16.5.2 Placental Changes in Gestational Diabetes

The detected histomorphological changes of the placenta in GDM vary considerably (Huynh et al. 2015a). The histomorphological changes shown in placentas of patients with gestational diabetes differ from those with pre-existing diabetes (Table 16.3). Here, increased placental weight (22%), increased diameter (33%) and thickness of the placenta (85%) are described in the first place (Ashfaq et al. 2005). This is accompanied by a decrease in the ratio of fetal weight to placental weight (Taricco et al. 2003). Studies that explicitly examined histomorphological criteria in gestational diabetes describe an increased incidence of necrosis and chorangiosis, infarcts and other signs of placental ischemia, and villous immaturity compared to healthy individuals (Daskalakis et al. 2008; Madazli et al. 2008). Severe structural changes, as in pre-existing diabetes, have rarely been reported.

Huynh et al. (2015a) compared the placentas of gestational diabetic women with those of patients with type 1 DM and type 2 DM in a histomorphological study (Table 16.3). They found a comparable

Table 16.3 Overview of histological changes found in the placenta in diabetes mellitus depending on the disease type (Huynh et al. 2015a)

Placental pathology	Pre-existing diabetes		Gestational diabetes
	Diabetes type 1	Diabetes type 2	
Growth/weight	Increased placental weight[aa]		Increased placental weight
Extent of villous maturation	Increased frequency of immature villi[a]		Increased frequency of immature villi
Angiogenesis	Increased number of capillaries in terminal villi[a] Increased average number of redundant connections per terminal villus[a] Greater combined length of fetal capillaries[a]		Increased average number of redundant connections per terminal villus[a]
Volume and surface areas of the parenchymal tissue[b]	Increased volume of tissue parenchyma[a]		Increased volume of tissue parenchyma[a]
Maternal vascular lesions (primary and secondary)	Increased incidence of nucleated fetal erythrocytes, fibrinoid necrosis, villous immaturity, and chorangiosis[a]		Increased incidence of fibrinoid necrosis and chorangiosis

[a] Findings compared to healthy controls
[b] Contains the structure and compartments affected by the metabolic exchange between mother and fetus

degree of signs of uteroplacental underperfusion. Placentas from patients with type 1 DM were less likely to have placental infarcts. Type 2 DM was more frequently associated with decidual vascular changes compared with GDM. In particular, the vascular-associated changes showed significant differences depending on the type of diabetes.

16.6 Placental Histology in Diabetes—Consequences of a Histopathological Reprocessing?

The clinical dilemma in patients with insulin-dependent diabetes in pregnancy is the obvious risk of sudden placental insufficiency with the danger of consecutive fetal death near term. Therefore, current guidelines worldwide continue to recommend induction of labor near term in patients with diabetes and insulin-dependent gestational diabetes (▶ Sect. 16.3.3). This applies regardless of the quality of metabolic control during pregnancy. For the mothers concerned, induction of labor is usually an undesirable measure that is subjectively perceived as deeply interfering with the physiological course of birth. An improved clinical selection of patients who benefit from induction is therefore desirable. To date, there are no prospective clinical studies on this subject that also stratify for metabolic control, sonographic parameters, and type of diabetes. These studies are also difficult to justify ethically due to the risk of intrauterine fetal death.

Two studies in recent years have already attempted to correlate histopathological changes in diabetic placentas with metabolic control in pregnancy. Rudge et al. compared the morphology of pregnancies with normoglycemia, mild GDM and latent DM. Compared with the normoglycemic group, there were significantly more signs of placental pathology in all groups, with no differences between the GDM groups but between these and the group of latent newly diagnosed diabetes (◘ Table 16.4) (Rudge et al. 2011).

In a recently published study, Starikov et al. correlated the histopathological changes in the placentas of patients with diabetes with the HbA1c in the first trimester. They found no correlation between the histopathological findings and blood glucose control at the time of placentation (Starikov et al. 2017).

The systematic histopathological reprocessing of placentas from pregnancies complicated by diabetes may contribute to a better understanding of the pathophysiological mechanisms underlying placental insufficiency. In this context, correlation of systematically collected tissue findings on the placenta with clinical pre- and postnatal data could provide evidence for an association between clinical parameters and expected placental dysfunction. In the long term, this could form the basis for prospective studies that could provide evidence to avoid induction of labor in certain clinical constellations where no change in placental morphology is expected.

Table 16.4 Number and percent of histopathological changes in the placenta in women with normoglycemia, mild GDM, GDM, or previously latent preexisting DM (Rudge et al. 2011)

Histopathological changes	Pregnancy groups			
	Normoglycemia	Mild GDM[a]	GDM	Open DM
Circulatory				
Cystoid degeneration		1 (2.9)	1 (12.5)	3 (3.6)
Chorial edema				2 (2.4)
Intima edema				4 (4.8)
Interstitial hemorrhage	1 (16.7)	2 (5.9)		3 (3.6)
Congestion	2 (33.3)	17 (50.0)	3 (37.5)	52 (62.6)
Subchorial infarction	2 (33.3)[b]	3 (8.8)[c]		3 (3.6)[c]
Degenerative				
Villous edema	1 (16.7)	7 (20.6)	3 (37.5)	18 (21.7)
Villous fibrosis	1 (16.7)	8 (23.5)		11 (13.2)
Intervillous fibrosis	3 (50.0)	28 (82.4)	6 (75.0)	65 (78.3)
Calcification	4 (66.7)[b]	15 (44.1)[b]	1 (12.5)[c]	42 (50.6)[b]
Focal hyaline degeneration	4 (66.7)	28 (82.4)	6 (75.0)	71 (85.5)
Proliferative				
Dysmaturity		5 (14.7)	3 (37.5)	8 (9.6)
Hofbauer hyperplasia				1 (1.2)
Chorangiosis	1 (16.7)	4 (11.8)		7 (8.4)
Syncytial knots	1 (16.7)[b]	11 (32.4)[c]		8 (9.6)[b]
Inflammatory				
Villitis				2 (2.4)
Focal amnionitis	1 (16.7)	4 (11.8)	1 (12.5)	7 (8.4)
Other				
Phantom cells		2 (5.9)		1 (1.2)
Two vessels		1 (2.9)		3 (3.6)
Endarteritis		8 (23.5)[b]	1 (12.5)[c]	7 (8.4)[c]
Double membrane	2 (33.3)[b]			1 (1.2)[c]
Umbilical cord bleeding	3 (50.0)	5 (14.7)		14 (18.1)
Total number	6	34	8	83

[a] *GDM* gestational diabetes
[b] Numbers represent a significant statistical difference ($p < 0.005$)

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Supplementary Information

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