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Pathology of the Human Placenta

Sixth Edition

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Preface

It is with great sadness that we report here that Professor Peter Kaufmann died in December 2010 from the cancer that had troubled him for a long time. Professor Graham Burton will ably replace Peter's contributions in the future.

Most obstetricians and pediatricians would agree that the examination of the placenta often helps to explain an abnormal neonatal outcome. As early as in 1892, Ballantyne wrote that:

> A diseased foetus without its placenta is an imperfect specimen, and a description of a foetal malady, unless accompanied by a notice of the placental condition, is incomplete. Deductions drawn from such a case cannot be considered as conclusive, for in the missing placenta or cord may have existed the cause of the disease and death. During intrauterine life the foetus, the membranes, the cord and the placenta form an organic whole, and disease of any part must react upon and affect the others.

Similar thoughts were succinctly detailed in Price's discussion of his concept of the "Prenatal Biases" as they affected twins. His contribution also admonishes us that placental study is a *sine qua non* for a more perfect understanding of fetal development (1950). Despite all this understanding of the past and appreciation for placental disease, great resistance still exists to perform the task of placental examination routinely. For many pathologists, therefore, the placenta has remained a mysterious organ.

This book had its beginning in 1967 when Shirley G. Driscoll and Kurt Benirschke wrote the volume on placental pathology for the *German Handbook of Pathology*, the *Henke-Lubarsch*. Because there seemed to be a need for wider dissemination of the text, this was reprinted by Springer-Verlag New York but soon became unavailable. Since then, a number of books on placental pathology have been written, in French, English, and German (Philippe, Baldwin, Fox, and Sebire; Perrin, Gruenwald, Lavery, Naeye, Becker, and Röckelein; Vogel, Kaplan, Joshi, and Baergen), and much more interest has been accorded to this "so readily available but poorly studied" organ. The journal *Placenta* founded by Harold Fox has become a significant outlet for results of sophisticated placental studies. The International Federation of Placenta Associations (IFPA) has been established to promote research interest and to integrate the activities of the former *Trophoblast Conferences* held in Rochester, N.Y., and the European and other regional placenta groups. Much new information has been obtained and the continuing enigma of placental non-rejection has been tackled by numerous investigators without complete resolution. In addition, the availability of the placenta for biochemical study has stimulated many cell biologists and molecular biologists to use this organ as a convenient source of human tissue. Genetic and epigenetic information now add to our understanding of the complexity of placental function, and so forth. Also, because much interest is developing in "Comparative Placentation," a website may be found at: http://medicine.ucsd.edu/cpa.

This sixth edition is being written because so many new findings have come from the systematic study in the last few years that updating seemed necessary. Moreover, there is a great need to have documentation for legal purposes as the placenta has become an important aspect of medico-legal adjudication of circumstances around the time of birth. The organization of the previous edition also left some topics uncovered that are now being corrected. Many changes have been made throughout the book. Not only has the text been updated, a more complete index has been created, the order of chapters is presented more logically and tables

are presented more usefully. The text was written with MS Word. A complete set of diskettes with the references can be made available from the authors, if desired.

I (KB) am indebted to many people, foremost to my wife for her understanding and patience with me and this task; the publishers with many of its people have been gracious and patient; my colleagues at the university; and other persons who have all helped gather data are gratefully acknowledged. Many students and colleagues have graciously read most chapters and they have made many helpful suggestions and corrections, for which I am appreciative. There are some colleagues, however, whose inspiration have helped more than others: Marjorie Grafe; the dysmorphologists Kenneth L. Jones and his wife Marilyn and their numerous fellows as well as neonatologist Frank Mannino; and ultrasonographer Dolores Pretorius who continues to challenge me and requires that I provide explanations for perinatal deaths and abnormalities. Having examined all placentas of all deliveries in the institutions with which I was affiliated over the past five decades, I have gathered a large amount of material to digest. Most of all, however, I am grateful to Dr. Geoffrey Altshuler, Oklahoma City, for many stimulating discussions and endless patience with me and his friendship.

PK gratefully acknowledges the scientific cooperation of many former and present coworkers. These comprise Mario Castellucci, Ayse Demir, Hans-Georg Frank, Hitoshi Funayama, Gabriele Gaus, Berthold Huppertz, Mahmed Kadirov, Sonja Kertschanska, Gaby Kohnen, Georg Kosanke, Azizbek Nanaev, Frank Reister, and the late Gertfried Schweikhart. Many of my data are based on their material, their findings, and their ideas. Also, many colleagues and friends from other laboratories have contributed by discussion and by offering technical help. In this respect I am particularly grateful to Ramazan Demir, Gernot Desoye, Jean-Michel Foidart, John Kingdom, Hubert Korr, Rudolf Leiser, Peter Ruck, Hobe Schröder, Tullia Todros, and the late Elizabeth Ramsey. In many cases it is virtually impossible to differentiate between their and my ideas. Unfortunately, since PK was taken ill he turned over the responsibility for his chapters to Professor Graham J. Burton of Cambridge, England. We are most grateful to him for accepting this task.

These chapters do not only require scientific inspiration but also much artistic, technical, and secretarial work. The artistic help of Wolfgang Graulich and the photographic assistance of Gaby Bock as well as of Helga Kriegel are gratefully acknowledged. The histological and electron-microscopic pictures are based on material processed by Marianne von Bentheim, Michaela Nicolau, Lian Shen, Barbara Ihnow, and Uta Zahn. Perfect secretarial assistance was provided by Jutta Ruppert. The collaboration of all these coworkers and friends was the basis for my contribution (PK).

I (GJB) wish to acknowledge the enormous contribution made by all members of my laboratory over several decades of placental research. I was introduced to the placenta by Donald Steven, and have had the great pleasure and privilege to work with many academic colleagues, post-doctoral fellows, and students. In particular, I am most grateful to Eric Jauniaux, Stephen Charnock-Jones, Ashley Moffett, Carolyn Jones, and Jeremy Skepper for long and productive collaborations, and for their comments on draft versions of chapters. I also wish to acknowledge the visionary philanthropy of Charlie Loke in endowing the Centre for Trophoblast Research (www.trophoblast.cam.ac.uk), which has provided a unique foundation for placental research.

I (RB) am most grateful to my husband for his patience and support. I am also grateful to a number of colleagues for their helpful suggestions and their inspiration including Ona Faye-Petersen, Debra Heller, and Cynthia Kaplan. I also would like to thank our residents and Pathology Assistants who have assisted with photographs and gathering data, but I would like to particularly thank our fellow Kristina Loukeris for her support and assistance which she graciously provided on a daily basis.

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Contents

Examination of the Placenta

1

1.1 Macroscopic Examination

Most placentas are normal, as are most babies. Therefore, an examination of all placentas may not be warranted, although this has been advocated repeatedly. Practical guidelines, including indications for the examination, have been published by the College of American Pathologists (Langston et al. 1997). This reference describes in tabular form the major abnormalities and their association with clinical features. Booth et al. (1997) inquired what reasons constituted the submission of a placenta for examination and found, regrettably, that it was Cesarean section delivery. This is hardly a good reason, as will be seen. A large number of surgical deliveries are repeat sections and have little impact on perinatal problems for which placental examination might be useful. Altshuler and Hyde (1996), on the other hand, found that 92% of placentas for which an examination was requested by an obstetrician or neonatologist had relevant pathology. Salafia and Vintzileos (1990) made a strong plea for the study of all placentas by pathologists. We concur with this view, as the sporadic examination does not provide sufficient training for young pathologists and it does not allow the "routine" pathologist to obtain sufficient background knowledge as to what constitutes a truly normal placenta. Another reason for the examination of all placentas is today's litigious climate; it makes study of placentas highly desirable (see Chap. 27). Furthermore, it has been shown repeatedly that a placental examination is needed to understand the causes of perinatal deaths. This was demonstrated, especially for stillbirths, by the study of Las Heras et al. (1994). The most important lesions were found in the umbilical cord (18%), with inflammatory lesions being second. Altshuler (1999) wrote a searching essay on the "placenta-related epidemiology" from his vast experience in these matters. Because placentas differ widely in shape, size, and in appearance, the novice must become familiar with this spectrum of placental shapes. To do so, a large number of placentas must be examined routinely. In hospitals with large numbers of deliveries, however, it may be prudent to select placentas for examination by the pathologist.

1.2 Storage

To facilitate the practice of saving placentas for a week, storage is required so that placentas are available when needed. The American College of Obstetricians and Gynecologists, on the other hand, has suggested, surprisingly, that the routine study of the placenta is not warranted (ACOG 1991), a decision with which we strongly disagree. Placentas should **not** be frozen prior to examination, as this obliterates the most useful histological characteristics and makes even the macroscopic examination more difficult. We believe that formalin fixation has a similar unwanted effect. It is best to store the delivered placentas in plastic containers. These containers can also be readily labeled and stored in a refrigerator at 4°C. In this state, the placenta is preserved for a meaningful examination for many days. Autolysis is minimal. We cannot agree with the opinion of Naeye (1987) that this storage causes significant artifacts at the gross level that render a subsequent examination difficult. Indeed, the immediate fixation of the organ in formalin, recommended by others (Bartholomew et al. 1961) as a good means to evaluate the extent of infarction, makes the placenta more difficult to evaluate critically, aside from the storage problems, expense, and odor. Prior fixation, of course, also makes tissue culture, bacteriologic examination, and other procedures more difficult or impossible. For maximal convenience, it is a good idea to have a refrigerator with seven shelves, labeled Monday through Sunday, and to discard the normal placentas from one shelf when the next similar weekday arrives. In this way, all placentas from problem births will be available for study.

The placenta loses some weight during storage. In part, the loss is due to evaporation, but most weight is lost by leakage of blood and serum occasioned by the mass of placental

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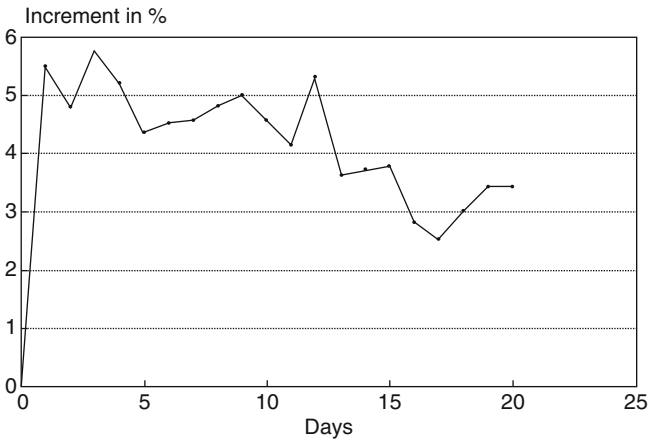

Fig. 1.1 Weight gain of placenta (trimmed, without cord or membranes) after formalin fixation

tissue resting on other portions. The amount of weight loss depends on the length of storage and the degree of edema, but it is not great in the normal placenta. It is most significant in the edematous placentas of hydrops. We have observed a 180-g fluid extravasation from a 740-g placenta within 1 day from a hydropic placenta. The freshly examined placenta is thus softer, bloodier, and thicker than one that has been stored. On the other hand, it must be noted that the placenta gains weight when it is stored in formalin, particularly during the first day of fixation. Not all organs increase in weight uniformly after such fixation, as the detailed report by Schremmer (1967) specified. The placenta, according to this author, gains between 0.7% and 23.0%, with an average of +9.9%. It is among the organs with the largest deviations in weight gain after fixation. Our own findings are summarized in the graph shown in Fig. 1.1.

1.3 Selection

Placentas from all prematurely delivered infants and all twins should be examined routinely, at least macroscopically, and many of them require histological study as well. In addition, many circumstances arise during the first few days of life of an infant where the neonatologist is interested in placental findings. These often help to clarify whether a particular disease had a prenatal onset. Furthermore, there are some maternal conditions that warrant placental examination, e.g., preeclampsia, the condition known as lupus anticoagulant, diabetes, fever, and many more. In our routine study of placentas, the obstetricians and neonatologists alert us as to which placentas they believe warrant more scrutiny, and thus, perhaps 5–10% of all placentas undergo histological examination in our hands.

1.4 Photography

A photographic record is often desirable and is useful for many purposes. Nowadays, of course, digital photography has become such a routine procedure, and storage of the digital images has become so easy that much more photography of specimens is desirable and practiced. The photographic task is generally quickly accomplished. Friends have often been amused by this recommendation, but they agree that a good picture is worth a lot of words, especially when it comes to litigation.

1.5 Examination

Detailed protocols for the examination of the placenta have been presented in the past (Benirschke 1961a, b; Gruenwald 1964; Fox 1997). Some protocols were designed to allow an unbiased examination of the placenta and record keeping by the many different medical centers of the "Collaborative Study" so that, ultimately, correlations could be made regarding fetal outcome. Our routine procedure now is to select for histological study those that appear abnormal or whose perinatal circumstances demand such an examination. The selection process just outlined has been helpful, and we have rarely missed a placenta that was of importance. Other recommendations for a "triage" of placental study and other ramifications come from a joint conference held in 1990 (Travers and Schmidt 1991). That volume provides useful information on many aspects of placental pathology.

The tools for the examination are simple (Fig. 1.2). They consist of a ruler, a long and sharp knife, toothed forceps, a pair of scissors, and a scale. Our ruler is permanently mounted over the cutting board, thus enabling rapid measurement of the length of the umbilical cord and the placenta's diameter. A butcher's scale with removable bucket that weighs items up to 2 kg is also available. The long knife, best obtained from a butcher supply house, is sharpened just before examination.

When the placenta is removed from its container, one often perceives rather characteristic odors. For instance, when a mother has recently eaten garlic, the intense smell of its diallyl sulfides is readily apparent. Also, in infected placentas, the fetid smell of *Escherichia coli* and the rather sweeter smell of *Listeria monocytogenes* can be distinguished by an experienced pathologist. Storage in the refrigerator enhances the growth and hence the recognition of these organisms.

The shape of the placenta is then ascertained by stretching it flat on the cutting board. Is it round or oval? This may be assessed by measuring the length of the longest axis and then the longest length of the axis perpendicular to the first, or by

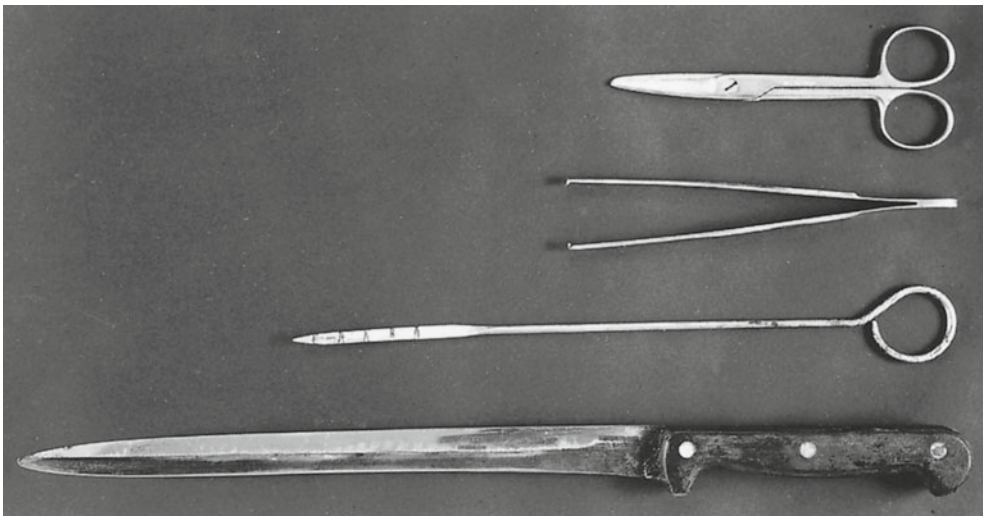

Fig. 1.2 Instruments found to be most practical for the placental examination: scissors, forceps, "dipstick" to measure the thickness of placental tissue, and a long, stiff knife

taking multiple measurements of the radius from the site of cord insertion (Pathak et al. 2010; Salafia et al. 2010). Depending on the methodology used, different conclusions are reached. Interest in placental shape has been revitalized by the recent observation that ellipticity is related to developmental programming of the fetus (Barker et al. 2010, 2011). The mechanisms underlying this association are unknown at present, but the shape of the placenta may reflect the site of implantation and hence potentially its maternal vascular supply, or some other aspect of uteroplacental physiology that impacts on placental efficiency.

Another feature to note is the presence of accessory (succenturiate) lobes. One finds that, during the delivery, the membranes have generally inverted over the maternal surface ("Schultze" procedure) and rarely are they found in the position they held *in utero* ("Duncan") (Pritchard et al. 1985). They are then inverted by the examiner so that they assume the *in utero* configuration, and one next ascertains the completeness of the membranes. It is also noted at this time if the tear that allowed the infant to escape from its membranous enclosure extends to the edge of the placenta or if free membranes extend beyond the edge. If there is any margin of intact membranes, this placenta could not have been a placenta previa, provided it was from a vaginal delivery. If the edge of the membranous tear is far from the placental border (often the case with circumvallate placentas), a fundal position can be deduced. Torpin and Hart (1941) made the point that when the minimally disturbed sac is immersed in a bucket of water, the sac assumes the configuration of the uterus, and that its position before birth can be reasonably accurately determined by this study. At this time, it is prudent to inspect the color and appearance of the fetal surface of the placenta. Normally, it is shiny, and the subjacent blood is seen as a clear blue hue, particularly in the immature organ. When chorioamnionitis is present, the membranes become opaque by the interposition of leukocytes, and the surface usually loses its sheen. Greenish discoloration betrays either meconium (slimy) or hemosiderin deposition.

Next the membranes are cut off the edge of the placenta with the knife. If one anticipates making sections of the placenta for histological study, it is wise to follow a routine protocol for doing it, as it enhances subsequent interpretation. It is preferable to cut the membranes off in such a manner that one knows the point of rupture; then, when sections are made, the membrane roll is prepared in such a fashion that the point of rupture is in the center of the roll with the amnion inward (Fig. 1.3). This method of preparing a roll of membranes (the "jelly roll"), in order to obtain a maximum amount of membranes with decidua capsularis, was first described by Zeek and Assali (1950). In immature placentas, there may be a large amount of decidua, and it is often ragged. In more mature organs, the decidua atrophies and often it degenerates. Occasionally, one finds an intrauterine device in this decidua capsularis, usually at the edge of the placenta and associated with old clot and debris (Figs. 1.4 and 1.5). Frequently, there are areas of brown to green discoloration in the membranes that are from former hemorrhages, or they may have been induced by amniocentesis.

In many placentas that come from patients after labor, in contrast to those after Cesarean section, the amnion is disrupted or sheared off the underlying chorion. In fact, the amnion may be totally detached. Often, though, there is milky, white vernix caseosa that has dissected underneath the amnion; it is readily moved about by pressure. It has no significance. Moreover, the membranes near the edge of the placenta frequently contain the remnant of the yolk sac, a small white to yellow oval disk that is located underneath the amnion. The yolk sac of early stages of development can now be visualized

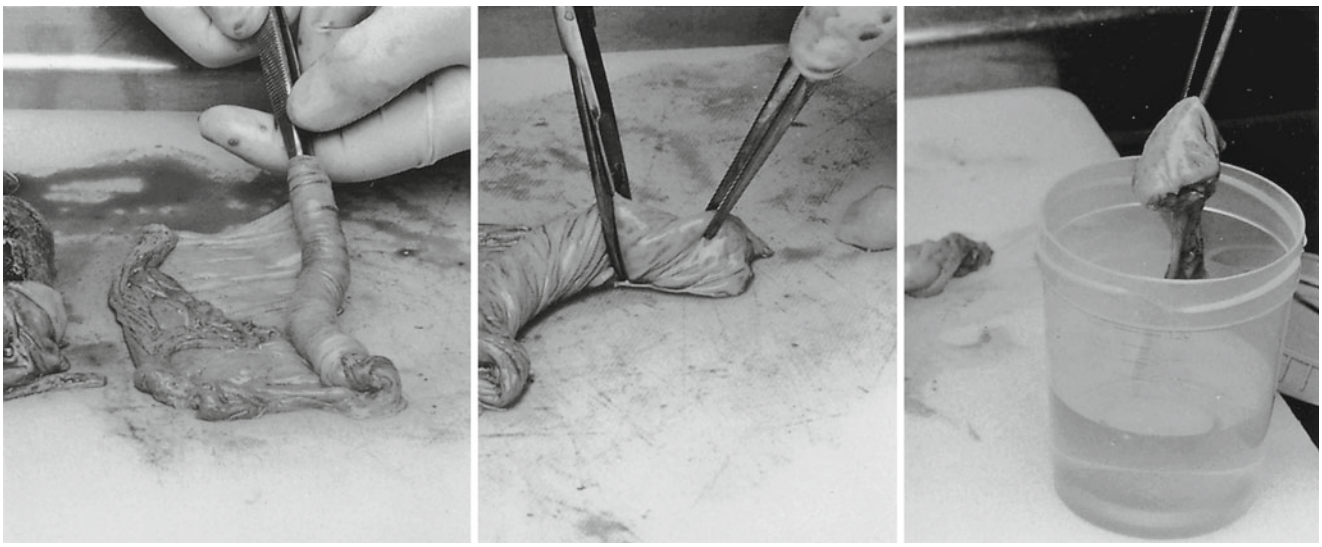

Fig. 1.3 Rolling of membranes for fixation and later sectioning. It is best to prepare them in a standardized fashion, e.g., amnion inside, starting at the site of rupture and proceeding toward the edge of the placenta, as shown at *left*. A segment is then taken from a well-rolled portion (*center*) and is fixed for a day (*right*) before trimming

Fig. 1.4 Edge of the placenta (*right*) with an intrauterine device embedded in degenerating decidua (partly removed) and old blood clot

ultrasonographically. Measurements have shown that the size of the yolk sac is variable, and that it is not a useful prognosticator for fetal well-being (Reece et al. 1988). Occasionally, one sees remnants of tiny vessels traversing from it to the insertion of the cord, or even within the cord.

The color of the membranes is noted, as are the surface characteristics. A slimy feeling is often the result of meconium discharge, as is of course a green color. The length of time of meconium discharge can be estimated by the presence of green discoloration in different layers. When it is only in the amnion, this suggests a short-time interval; when meconium is found also in the chorion after the amnion is stripped off, a longer interval has passed since discharge (Miller et al. 1985). We found that after 1 h, the meconium macrophages are visible within the amnion; after 3 h, they may be seen in the chorionic membrane. At even later times, it reaches the decidua capsularis. Greenish or brownish discolorations in immature placentas are more often due to blood breakdown products (hematoidin, hemosiderin) following hemolysis, rather than due to meconium. Hemosiderin, of course, can be stained with the Prussian blue method for iron, and the bilirubin of meconium stains (poorly) with bile stains. The very immature fetus cannot discharge meconium, lacking the hormonal maturation for intestinal propulsion (Lucas et al. 1979). The surface of the membranes, the amnion, is normally shiny. Around the insertion of the cord, one may find squamous metaplasia in the form of concentric nodules that are hydrophobic (Fig. 1.6). They are normal features. Amnion nodosum, usually represented by a finely granular, dull appearance of the amnionic surface, correlates with oligohydramnios. One must, of course, be cognizant of whether the amnion is present at all and, if not, whether amnionic bands exist. Also, often some blood has dissected underneath the amnion during delivery or, especially, when fetal blood has been aspirated for diagnostic tests from the fetal surface blood vessels.

Then, the cord is examined; is it central, eccentric, marginal, or membranous (velamentous) in its insertion? What is its length, and is it spiraled? We now believe that the length of the umbilical cord is determined primarily by fetal movements and that excessive spiraling implies unusual fetal motions (Moessinger et al. 1982). There may be a genetic component to the spiraling and the length, as the umbilical cords of some animals have different and consistent lengths, but this characteristic is so far unknown for human umbilical cords. Are there knots, thrombi, or discolorations? Can any

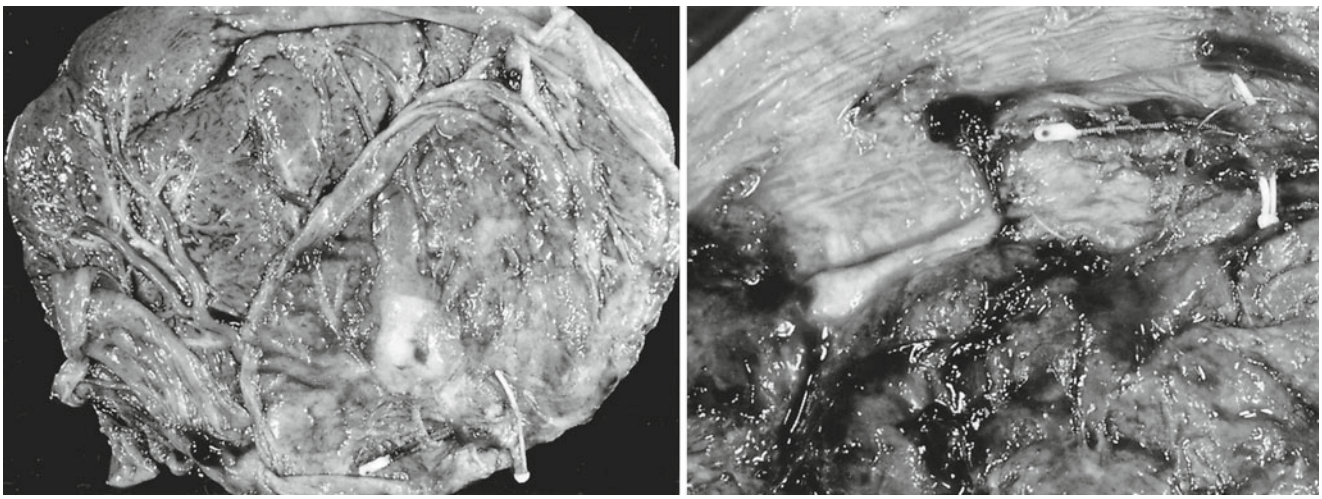

Fig. 1.5 Intrauterine devices at the placental margin at term (*left*) and in a slightly immature (*right*) pregnancy. Note the attending hemorrhagic degeneration of the adjacent tissues

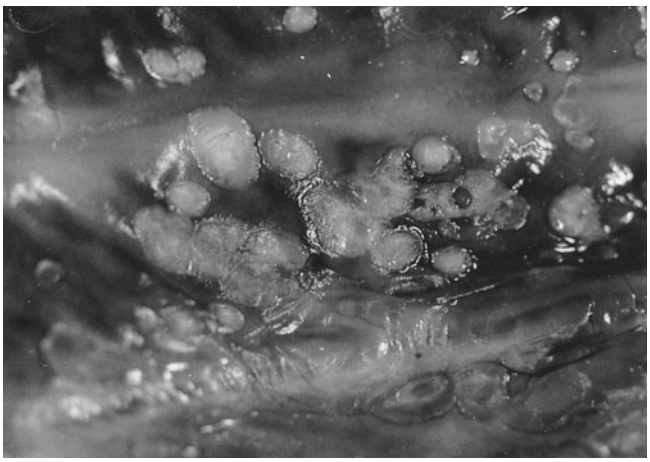

Fig. 1.6 Squamous metaplasia of amnion in concentric patches, usually found near the insertion of the umbilical cord. The plaques are water repellent

other unusual features be detected? The cord is then severed from the bulk of the placenta, and its cut surface is studied at several locations. The most important observation to be made here is whether there are three vessels and if other unusual features are present. Single umbilical artery (SUA) is the commonest abnormality. One must also appreciate that there is almost always an anastomosis ("Hyrtl's anastomosis") between the two umbilical arteries which is usually found near the point of insertion on the placental surface (Priman 1959). Thus, counting the number of vessels is best done farther away from the insertion. When a velamentous insertion of the cord is found, the examiner must pursue the ramifications of the fetal vessels after they leave the site of cord insertion, at times finding thrombi, and particularly in membranous vessels. These vessels may be disrupted, as in vasa previa, and acute exsanguination of the fetus is common in such circumstances.

The weight of the remaining disk is ascertained. It is generally useless to know the weight of the entire organ, including cord and membranes. Correlations with fetal weight and development can be made only by knowing the "net" weight of placental tissue (Walker 1954; Gruenwald and Minh 1961). Excessive amounts of maternal, retroplacental clots must, of course, also have been removed before weighing. Note again that the weight of formalin-fixed placentas is greater than that of fresh organs (Fig. 1.1) (Schremmer 1967). Variations in normal placental weight are common. They reflect mostly length of storage and amount of fetal blood content.

When studying the fetal surface of the placenta, one notes its color and the possible presence of granular excrescences. Most importantly, however, one must carefully inspect the fetal vessels, which are carried in the chorion; the amnion has no blood vessels. In nearly all placentas, one can recognize the fetal arteries as those vessels that cross over the veins (Boe 1953; Crawford 1962). It will be observed that the terminal branches of arteries dip singly into a lobule; next to it, a vein emerges to return the blood to the cord (Fig. 1.7). One often finds thrombi in these vessels in placentas of abnormal newborns. They appear as white-yellow streaks on the vessel's surface and are usually not completely occlusive. When they are, an area of hemolysis is often seen adjacent to the thrombosis. Thrombi may also calcify. They must be sampled for histological study.

The fetal surface of the mature placenta is often described as being "bosselated" or "tessellated," meaning that tiny white elevations are present underneath the chorion, giving the surface a mosaic, irregular pattern. These protrusions represent accumulations of fibrin in the intervillous space,

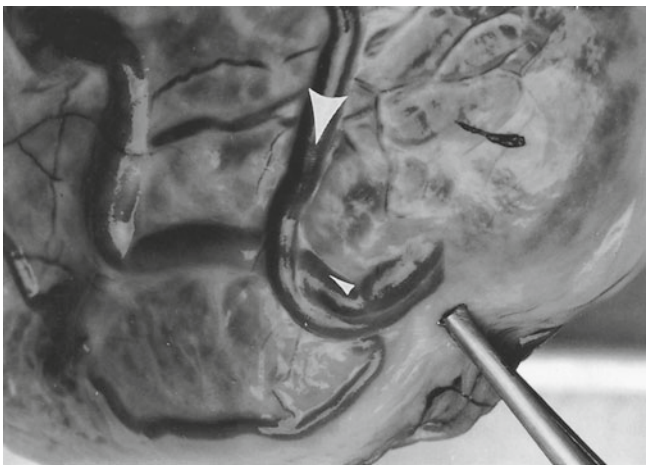

Fig. 1.7 Entrance of fetal vessels on the chorionic plate into the cotyledon. One artery (*large arrowhead*) brings the fetal blood; the vein (*small arrowhead*) next to it returns it to the fetus

and they increase in number with advancing maturity. Larger patches of fibrin also exist; at times they have a liquefied center, but they are assumed to be of little significance (Geller 1959). In our experience, however, larger subchorionic thrombi are abnormal and occasionally associated with fetal growth restriction. Cysts from the subchorionic extravillous trophoblast cells ("X-cells") may bulge on the surface and contain a clear, slightly viscid mucoid substance. At times it is discolored with blood. Finally, the insertion of the membranes is observed. Was it at the edge, or was there a ring of "circumvallation"?

When the placenta is turned over then, thus exposing the maternal surface, the first need is to identify possible areas of abruptio placentae. When an abruption is fresh, one may not be able to differentiate it from the normally present postpartum maternal blood clot that adheres. Within a few hours, though, the blood dries, becomes firmer and stringy, and then changes color to brown, and eventually it may become greenish. In such cases, the placenta underneath the clot is usually infarcted, or it is at least compressed. Abruptios are common, and most are clinically silent. Most are located at the margin of the placenta; and on occasion, one finds an old clot behind the membranes. Calcification on the maternal surface is then sought: small yellow-white granules in the decidua basalis and septa. Calcifications vary a good deal in quantity; they are usually found only in mature organs. The quantity has no clinically important correlations (Fujikura 1963a, b; Jeacock et al. 1963), but clinicians have paid much attention to the recognition of calcification. It may be detected by sonography and has served as a method to "grade" (age) the placenta (Fisher et al. 1976; Grannum et al. 1979). This is rarely done now as it has not been found to be helpful. At this point, one also observes the cotyledons, the major subdivisions of the placental tissue. They increase in size and differentiation with advancing gestation, being absent early. One needs to ascertain now whether all of the placental "floor" is present or whether there are missing cotyledons. If no cotyledonary subdivisions exist in the mature placenta, then the floor is often too thick and it may be infiltrated with an excess amount of fibrin. This condition is known as "maternal floor infarction" (MFI) and is best noted at this time (Naeye 1985).

Long, parallel cuts are now made with the long knife and, most importantly, the color of the villous tissue is observed. The red color of the villous tissue is almost wholly determined by its content of fetal blood. Thus, a congested placenta (as in maternal diabetes, for instance) is dark, that of an anemic, hydropic, exsanguinated, or erythroblastotic fetus is pale, and it is usually also much more friable. Such a placenta is also commonly thicker, 3–5 cm, in contrast to the normal placenta that averages 2.0–2.5 cm at term.

It is normal to find "holes" in the center of many placental cotyledons (Schuhmann 1982). Such holes were filled, in vivo, with maternal blood and represent the areas of first blood distribution into the intervillous space from the maternal injection jet. Intervillous thrombi, often located in these spaces, may be dark when fresh; alternatively, they are composed of layered white fibrin when older. The intervillous thrombi differ from infarcts in that they displace villous tissue. Furthermore, infarcts are granular in contrast, because they are composed of dead villous tissue. Fresh infarcts are red, and older ones are yellow to white. When sectioning the placenta, one also finds that the intercotyledonary septa contain some calcium, and often they contain some cystic spaces filled with trophoblastic secretion, the same clear mucoid material as contained in surface cysts. They too arise from extravillous trophoblast cells. Occasionally, one encounters round tumors of a solid nature, chorioangiomas. It is a good practice to estimate the total amount of infarction and to record it; in fact, it is ultimately of some importance and may have medicolegal implications in infants with growth retardation. Single marginal infarcts are common and do not correlate with either fetal or maternal conditions. Other lesions are occasionally seen. Thus, some lesions that appear grossly as "infarcts" may turn out to be choriocarcinoma on histological study (Driscoll 1963).

1.6 Placentas of Multiple Births

Placentas of multiple births are important records for the infants and pediatrician alike, and they are routinely examined. A recording of the membrane relation between the twins, triplets, and so on is mandatory. Of course, for meaningful analysis, it is necessary that the umbilical cords be labeled with sutures or clamps by the obstetrician, in the

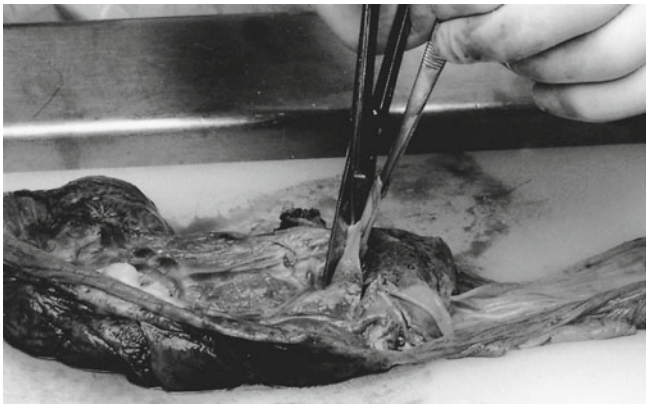

Fig. 1.8 Preparation of a "T section" of the meeting point of the dividing membranes in twin placentas

order of births. The most important decisions to be made in examining placentas of multiple births are (1) the number of membranes that divide the sacs (two or four) and (2) the types of vascular anastomoses that are generally present only in monochorionic twin placentas. Fraternal (dizygotic) twins essentially *always* have diamnionic/dichorionic (DiDi) placentas. Fused placentas, however, are not always monochorionic. They may be DiDi, and they may be diamnionic/monochorionic (DiMo). Finally, there may be no "dividing membranes" between the fetuses, as in the monoamnionic/monochorionic twin placenta (MoMo). All monochorionic twin placentas belong to monozygotic (MZ, "identical") twins. The time at which MZ twins separated one from another during the early embryonic stages presumably determines the type of placentation that ultimately develops, and this can thus be estimated from an examination of the membrane relation. It is easiest, but not necessarily best, to separate the "dividing" membranes from each other. If there are four distinctive leaves, it is a DiDi placenta, whereas if only two membranes are apposed, it is a DiMo placenta. Equally readily, the diagnosis of a DiDi twin placenta is made by ascertaining that the dividing membranes are opaque and contain remnants of old vessels or other debris (old decidua, degenerated villi). Also, in DiDi placentas, one usually finds a ridge at the site where the membranes meet over the placenta. It is caused by the buckling of tissues from the collision of the two expanding placental tissue masses. The dividing membranes of DiMo placentas, in contrast, are transparent. The diagnosis of membrane relationship is, of course, easiest and most permanently established by a histological section of a membrane roll of this tissue, or by a "T section" that includes this area (Fig. 1.8).

The location of the cord insertion is especially important in twin placentas, as it is much more frequently marginal or membranous than in singletons; this may reflect some problems in early placental development. Moreover, the absence of one umbilical artery (single umbilical artery – SUA) is more common in multiple births (Heifetz 1984).

After the membrane relation is established, the "vascular equator," i.e., the area where the two chorionic vascular districts meet, is examined. In DiDi placentas, there is never confluence of fetal blood vessels; if one were found, it would be exceptional and would be the basis for the exceedingly rare blood chimerism in fraternal twins. It must be cautioned here that ascertainment of a DiDi relation does *not* make the diagnosis of fraternal twins. Approximately one third of identical twins have this placentation (discussed in greater detail twin Chap. 25). In monochorionic placentas (DiMo, MoMo), there are almost always some anastomoses, particularly in the prematurely delivered placentas. These anastomoses have a great influence on the well-being of the developing fetus (Benirschke 1961b; Bejar et al. 1988). They take three forms: artery to artery (AA), vein to vein (VV), and artery to vein (AV). The last of these is doubtless the most important and is the basis for the "twin-to-twin transfusion syndrome (TTTS)." It must here be remembered that arteries lie on top of veins and that they are thus readily identified macroscopically. An A-V anastomosis carries the blood of one twin, through a cotyledon in a one-way direction, from one twin to the other. Often the various types of anastomoses coexist, and the consequences for fetal development may be different depending on the arrangements that are present. When in doubt, one injects the vessels in question with colored water or milk, all being readily available in obstetrical suites. Only the most sophisticated studies require injection with plastics (Panigel 1962). Injection of vessels has presented some problems for novices. First, it must be remembered that most placentas have suffered some disruption during delivery, especially the immature twin placentas. Thus, only small, selected districts should be injected, and this should be done only after the umbilical cords have been cut off in order to reduce resistance. Before injecting, one should seek to identify by careful inspection those areas that seem most "profitable" for the injection study. Large interarterial anastomoses (common) may be identified by one's ability to push blood back and forth from one side to the other. When one attempts to demonstrate the areas that reflect shared cotyledons, as in the transfusion syndrome, it is best to use a 20-mL syringe and a large (15-gauge) blunt needle. This is inserted into an arterial branch a little away from the prospective site and gradually one then fills the area with water or milk. The cotyledon will first rise, and when completely filled, it will empty into the vein that drains the cotyledon. It is also advisable to make a drawing or photograph of the anastomotic arrangements among multiple placental vascular districts, just to have them available for the record.

Examination of the maternal surface and other parameters of the placentas of twins follow that of the regular protocol.

It must be borne in mind that when the blood content of twins differs considerably, it may be reflected in the macroscopic placental examination as well. One portion of such a twin placenta may be severely congested and larger, with the other being pale and smaller. This condition is present when only one A-V anastomosis exists. Here, in the classical mechanism of the transfusion syndrome, one twin constantly loses blood through this one-way A-V shunt, whereas the other becomes plethoric. Usually, it leads to hydramnios, premature birth, and disparate birth weights of these "identical twins." It must be recognized, however, that differences in neonatal hemoglobin content of monochorionic twins may also occur acutely, when large AA and VV anastomoses exist. Thus, after the delivery of one twin, the other twin may "bleed" through anastomoses if the cord of the delivered twin is not promptly clamped. Likewise, when one of such twins dies *in utero*, significant shifts of blood may occur from the live twin through such large anastomoses into the relaxed vascular bed of the deceased twin. Finally, it is our practice to dissect the two halves of the twin placenta at the site of the vascular equator in order to determine the placental weight of each twin. Higher multiple births are handled the same way.

1.7 Fixation

The pathologist is used to fixing tissues for histological study in 10% formalin solution (a 1:10 dilution of the commercial 40% formaldehyde), and there is no need to make an exception with the placenta. For routine histopathology, we prefer Bouin's solution, however, because it makes embryonic and placental tissue considerably harder and allows one to trim the tissue more readily before embedding. After a membrane roll is made with the help of the forceps, it is also much easier to trim this jelly roll when Bouin's solution, rather than formalin, has been used. Bouin's solution is made by preparing a saturated solution (1.2%) of picric acid in water and adding 40% formaldehyde solution and glacial acetic acid in proportions of 15:5:1. After overnight fixation, the tissue is ready to be trimmed. The solution has the additional benefit of decalcifying fetal tissues. Prolonged storage of tissues in Bouin's solution makes them very hard.

Carnoy's solution is a useful alternative if immediate fixation is required directly after delivery and if obstetricians refuse the use of formaldehyde in the vicinity of the delivery room. This fixative is composed of 60 mL absolute ethanol, 30 mL chloroform, and 10 mL glacial acetic acid. It guarantees good structural preservation, provided that the thickness of the tissue blocks does not exceed 3–4 mm.

Many other fixatives have been used. Jiricka and Preslickova (1974) made a detailed study of seven solutions and evaluated the effect for the staining characteristics with different dyes. They found that none is ideal for all purposes, so the fixative must be chosen that gives the best results for a specific reason. The authors presented this information in tabular form, and the paper must be consulted if optimal results are to be obtained.

Ideally, the sectioned slices, when Bouin-fixed, are immersed in a saturated lithium carbonate solution before embedding. This step is not absolutely required, but it helps to remove extraneous pigments. Moreover, some intervillous blood is lysed, and pigments derived from blood ("formalin pigment," acid hematin) are more frequently present when lithium carbonate is omitted. Note, however, that occasionally the use of Bouin's fixation is disadvantageous. For instance, Altshuler and Hyde (1985) reported that infection with fusobacteria was less readily appreciated after Bouin's fixation than when formalin was used. Furthermore, Bouin's solution is not useful for fixation when the purpose is to conduct immunohistochemical or *in situ* hybridization studies (Gleich GJ, 1989, personal communication). Today, many immunohistochemical studies can be carried out on paraffin sections, such as the demonstration of most cytoskeletal proteins, extracellular matrix molecules, and several proliferation and apoptotic markers. For all these purposes, we suggest the fixation in 4% neutral buffered formaldehyde solution for a maximum of 24 h followed by paraffin embedding not exceeding 60°C. Antigen retrieval may be necessary, depending on the antibody being used, but care must be taken when interpreting the data as excessive use of the techniques may lead to a loss of specificity. Possible flow cytometry is also more readily done with such material.

It is the recommended practice to save at least one section of umbilical cord, a membrane roll, and three pieces of placental tissue for histological examination. Of course, having more sections of umbilical cord available for histological study is ideal, as an inflammatory response, thrombi, and other features are not always uniformly distributed throughout the length of the umbilical cord. Preparing more than one piece of placental tissue for histological study is also desirable because so many areas of the placenta show histological variations. Thus, one can much better determine the existence of inflammatory lesions and is less apt to overlook changes that are not ascertained macroscopically. Moreover, one must obtain sections from the more normal portions of the placenta as well. Although the pathologist is used to sampling abnormal areas for histological study, it is not desirable to take only abnormal areas of the placenta. Indeed, almost all infarcts are histologically alike, and since they also have a typical macroscopic appearance, they are rarely worth the trouble of histological study, except that the sections provide verifiable evidence of the existence of infarcts. It is much more important to save normal appearing placental tissue for microscopy. One must sample both the fetal and maternal surfaces in order to include some fetal surface blood vessels. Because it is generally impossible to anticipate from

macroscopic inspection whether chronic villitis and many other lesions exist, it is better to preserve too much than too little in the fixative. It goes without saying that unusual-appearing areas must also be sampled.

For histological examination, we prefer the hematoxylin and eosin (H&E) stain. On many occasions, however, it is useful to employ special stains, such as those that demonstrate elastic fibers, bacterial and spirochete stains, periodic acid-Schiff (PAS) preparations, and specific immunohistochemical stains that disclose the presence of viruses, e.g., cytomegalovirus and herpes antigens, as well as specific proteins, e.g., human chorionic gonadotropin (hCG), human placental lactogen (hPL), major basic protein (MBP), cytokeratin, vimentin, fibrin, and proliferation markers. These tests have given much insight into the distribution of various placental tissue components, the sites of hormone production and the involvement by organisms as well as other pathological processes. The report form used by us during placental examination is reproduced at the end of the chapter for the benefit of the reader.

1.8 Stereological Analysis

Quantitative assessment of placental structure is being used increasingly as a research tool, enabling objective data describing, for example, normal placental development to be obtained (Simpson et al. 1992), or comparisons to be made between pathological conditions such as intrauterine growth restriction, with and without preeclampsia (Mayhew 2009). Since the circulatory systems of the placenta collapse following delivery, and this has a profound impact on the resultant measurements, it is important that collection and handling of these placentas is performed in as standardized way as possible. Thus, factors such as time and mode of clamping of the cord (Bouw et al. 1976), and the time between delivery and sampling (Feneley and Burton 1991) must be kept as constant as possible. Even so, the dimensions of the fetal vessels will always be less in a delivered placenta compared to the in vivo situation. To avoid this complication, perfusion fixation under physiological pressures has been employed (Burton et al. 1987; Larsen et al. 2002), but this may not always be practicable within a clinical setting.

When it comes to sampling the placenta, there are two general strategies: to sample from defined sites for the purpose of making *a priori* comparisons, and global sampling to obtain data representative of the entire organ (Mayhew and Burton 1988). The sites for the first will be determined by the question being addressed. For the second, a systematic uniform random sampling method is best employed, and at least four blocks should be taken per placenta to account for regional variability (Mayhew 2008). The unbiased techniques applied will again depend on the questions being addressed, but the articles by Clausen et al. (1998) and Mayhew (2006) provide good overviews of the range.

1.9 mRNA and Microarray Analyses

Studies of gene expression have been used to investigate the pathophysiology of various placental disorders. The advent of commercially available microarray platforms allows thousands of transcripts to be analyzed at once, and for gene networks to be identified. However, special collection procedures are required if the data are to be representative of the in vivo state. These start with the mode of delivery. Placentas subjected to labor display higher levels of oxidative stress compared to nonlabored placentas delivered by Cesarean section, and this is associated with changes in the endocrine and cytokine profile. Many of the changes reported in preeclampsia are observed in normal placentas following labor (Cindrova-Davies et al. 2007). Ideally, therefore, analysis should be restricted to placentas delivered by Cesarean section. mRNA degrades rapidly following delivery, and tissue samples need to be frozen rapidly, preferably within 10 min. After this time, metabolic and oxidative changes are detectable (Serkova et al. 2003). Multiple small samples should be taken in a systematic random uniform fashion (Mayhew 2008) from at least ten placentas in each group, as studies have shown that there is large intra- and interplacental variability in gene expression (Pidoux et al. 2004; Avila et al. 2010). The samples should be washed thoroughly in buffered saline to remove maternal contaminants, frozen rapidly in liquid nitrogen, and then stored in a minus 80°C freezer for subsequent analysis. A suitable protocol has been described by Pasupathy et al. (2008).

Results from microarray studies should be confirmed by qRT-PCR techniques, and the general consensus is that three housekeeping genes should be used to increase the accuracy of normalization. Suitable housekeeping genes have been identified (Meller et al. 2005; Murthi et al. 2008).

1.10 Special Procedures

The placenta can serve as a good source of tissue for *chromosome analysis*. This is especially true when the fetus is macerated. One proceeds best by disinfecting the amnion with some alcohol and then stripping the amnion off a portion of placental surface. For the purpose of taking the biopsy from chorion, sterile instruments are recommended. A small piece of chorion, ideally with a bit of fetal surface vessel, is best for the purpose of establishing a tissue culture. The biopsies are placed into tissue culture medium with antibiotics and transferred to the laboratory. It is of parenthetic interest that Jauniaux and Campbell (1990) showed that many structural abnormalities of the placenta can be anticipated from sonography.

REPORT OF PLACENTAL EXAMINATION

NAME of PATIENT:..... Path. #.....
Unit #.....

HISTORY:.....
.....
.....
INFANT:.....
.....

Macroscopic:

WEIGHT (disk only).....g Formalin-fixed.....Fresh.....
SIZE.....×..... ×.....cm
CORD: INSERTION: Central..... Eccentric:.....cm from margin
Marginal....Velamentous.....Vasa previa.....
Vessels: 3...2... Thrombosis: Yes...No...Knots:.....Twists: Right…Left….
MEMBRANES: Marginal..... Circumvallate..... Color: Green.....Opaque.....Normal.....
Point of rupture from margin:.....cm
Amnion nodosum.....
SURFACE VESSELS:.....
TWINS: Yes....No..... HIGHER MULTIPLES:.....
DiDi.....DiMo.....MoMo....
Describe.....Anastomoses.....
MATERNAL SURFACE: Intact: Yes....No.... Calcification: Marked....normal....no...
Color:..... (Normal?).....(Pale?).....
Abruptio: Yes.... No..... Size.....cm Old.....Recent.....
CUT SURFACE: Infarct..... % of total placenta.... Old..... Recent....
TUMORS.....
OTHER.....

Microscopic:.....
.....
.....

DIAGNOSIS:

.....
Pathologist

Pictures taken? Yes.....(Color.....B&W.....Digital…..) No.....

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Macroscopic Features of the Delivered Placenta

2

The full-term, delivered placenta is, in more than 90% of the cases, a disk-like, flat, round to oval organ. In nearly 10%, it has abnormal shapes, such as placenta bilobata, placenta duplex, placenta succenturiata, and placenta membranacea (Torpin 1969). The average diameter is 22 cm, the average thickness in the center of the delivered organ 2.5 cm, and the average weight 470 g (see Appendix A.1). The respective measurements show considerable interindividual variation and strongly depend on such factors as the mode of birth, timing of cord clamping (see Appendix A.8), and time elapsed between delivery and examination.

2.1 Fetal Surface

The fetal (chorionic or amniotic) surface, facing the amniotic cavity, has a glossy appearance because of the intact epithelial surface of the amnion. This avascular membrane covers the chorionic plate, including the chorionic vessels. The latter branch in a star-like pattern centrifugally from the cord insertion over the fetal surface (Fig. 2.1a). Where arteries and veins cross, the arterial branches are usually superficial; they cross the veins on their amniotic aspect. Wentworth (1965) reported that only about 3% show the opposite condition. According to Boyd and Hamilton (1970), the superficial position of one or few venous branches at points of arteriovenous crossing is not unusual.

In the vicinity of the larger chorionic vessels, the chorionic plate normally has an opaque appearance because an increased number of collagen fibers accompany the vessels. Those areas of the chorionic plate located between the chorionic vessels are mostly transparent and are dark lilac to black because of the maternal blood in the intervillous space below. Opaque spots (bosselations) or large opaque areas independent of chorionic vessels usually point to large subchorionic deposits of Langhans' fibrinoid.

Near the placental margin, where the most peripheral branches of the chorionic vessels curve vertically toward the marginal villous trees, the transparency of the chorionic plate decreases, resulting in a largely incomplete, opaque, subchorial closing ring that is a result of increased amounts of cytotrophoblast and collagen fibers (see Chap. 9). It connects the placenta with the membranes. In the case of a particularly broad and prominent subchorial closing ring, the specimen is called a placenta marginata. A placenta circumvallata is formed when the closing ring is peripherally undergrown by villous trees. In such cases, it does not represent the outermost margin of the placenta; rather, the membranes insert superficially from the fetal surface of the placenta.

Placental shape and cord insertion are sometimes regarded as structurally impressive but functionally unimportant parameters. Whether the normal placenta is considered round or elliptical depends heavily on the algorithms used to derive a shape index. Taking multiple radial markers leads to the conclusion that the placenta is round (Salafia et al. 2010), whereas taking the longest and shortest dimensions perpendicular to each other results in the conclusion that it is elliptical (Pathak et al. 2010). Increased variability in shape is related to a decreased efficiency of the placenta, as assessed by the ratio of placental and fetal weights, which may reflect either maternal uteroplacental or fetoplacental pathology (Salafia et al. 2007, 2010). Due to the orientation of the blastocyst at the time of implantation, with the animal pole associated with the inner cell mass adhering to the uterine epithelium, the cord is normally inserted near the center of the disk. A recent large study revealed in fact that that the site of insertion is most commonly off-center (Pathak et al. 2010). Variations may therefore reflect aberrations in the initial process of attachment. Alternatively, it has been suggested that excessive villus regression, secondary to abnormal onset of the maternal arterial circulation toward the end of the first trimester, results in the cord being attached toward the margin of the remaining placental mass (Burton et al. 2010). Whatever the cause, eccentric insertion is associated with a lower fetoplacental weight ratio, again suggesting a less efficient placenta (Yampolsky et al. 2009). Whether this

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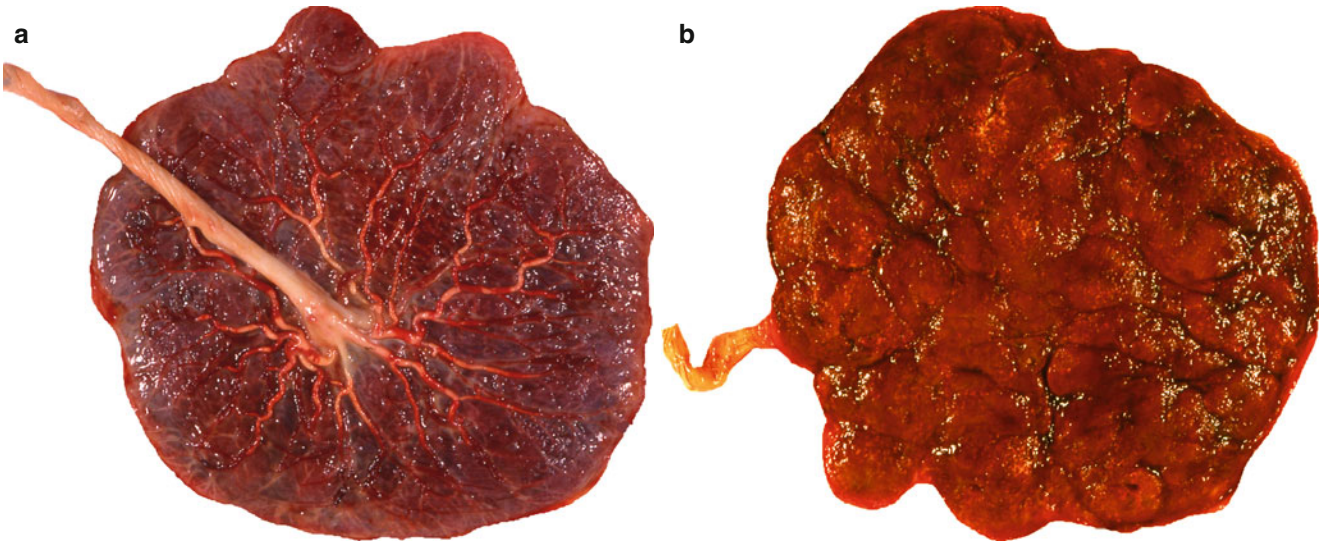

Fig. 2.1 Apical (**a**) and basal (**b**) views of a freshly delivered, mature human placenta. Note the slightly eccentric insertion of the umbilical cord, which is the most usual location. The chorionic arteries (*white* because of postpartum injection of milk) cross over the corresponding veins (*dark*). The basal surface (**b**) is subdivided into placental lobules of varying size by an interrupted net of dark grooves ×0.4

reflects compromise of the maternal vascular supply or a reduced exchange capacity on the fetal side is not known, but eccentric cord insertion is generally associated with a higher resistance in the umbilical circulation (Nordenvall et al. 1991).

2.2 Maternal Surface

The uterine (maternal) surface of the placenta is opaque, as it is an artificial surface originating from laminar degenerative processes within the junctional zone that led to the separation of the organ. This separation process subdivides the junctional zone between placenta and uterine wall into

- The basal plate which is attached to the placenta and represents the maternal, uterine surface of the organ
- The placental bed which remains in utero

The basal plate and the maternal surface of the placenta could not be identified before placental separation in the in situ specimens that were fixed before onset of labor (see Fig. 4.6). It is composed of a heterogeneous mixture of trophoblastic and decidual cells embedded into prevailing amounts of extracellular debris, fibrinoid, and blood clot.

An incomplete system of grooves subdivides the basal surface of the placenta into 10–40 slightly elevated areas called maternal lobes or cotyledons (Figs. 2.1b and 2.2). Internally, these grooves correspond to the placental septa, folds of the basal plate which project into the intervillous space (see Fig. 4.6). In histological sections, the septa can often be seen to be indented at their basal surfaces. It is likely that these grooves and the respective basal indentations of the septa are the postpartal results of tearing at sites of minor mechanical resistance, as the basal central parts of the septa are often characterized by necrotic zones, clefts, and local pseudocysts. Despite their possibly artifactual genesis, the grooves delineate the lobes and mark the position of the septa. As is described in Chap. 9, the septa must not be misunderstood as separating structures that subdivide the intervillous space into chambers; rather, they are irregular pillars or short sails that only trace the lobar borders.

The lobes show fairly good harmony with the position of the fetal lobules or cotyledons. From the chorionic plate at term, 60–70 villous stems arise, each branching into one villous tree (or lobule) (see Figs. 4.6 and 7.18). Thus, according to Boyd and Hamilton (1970) and Kaufmann (1985), each lobe is occupied by one or several villous trees. When a radioangiograph of the villous trees is projected onto a basal view of the same placenta (Fig. 2.2), the borderlines of the lobes usually coincide with the borderlines of single villous trees or small groups of trees. Small marginal lobes are likely to be occupied by only a single villous tree and thus correspond to what Schuhmann (1981) and his group described as representing a placentone.

2.3 The Terms "Fetal Placenta" and "Maternal Placenta"

When describing human placentation, terms such as "fetal placenta" and "maternal placenta" must be avoided because they are misleading and often cause misinterpretation. The terms originate from study of the noninvasive placentas of many domestic species, where a fetal component interacts

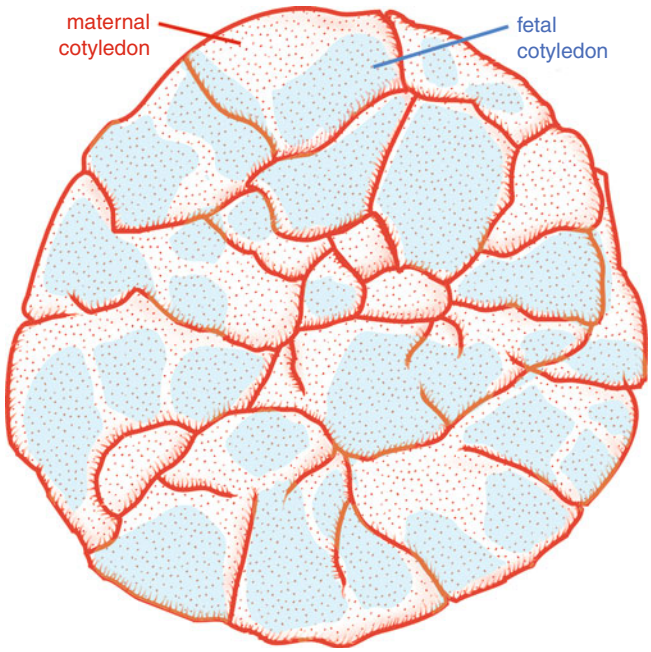

Fig. 2.2 Basal view of the placenta, drawn in combination with a radiograph of the same placenta after injection of a radiopaque medium into the fetal vessels. The borderlines of the placental lobules (maternal cotyledons, *red stippled*) are marked by *red lines* corresponding to the grooves. The radiographic projections of 29 villous trees are represented *by blue stippled areas*. This combination demonstrates a fairly good harmony of villous trees and maternal lobes. One to three villous trees (fetal cotyledons) are projected on one lobe (maternal cotyledon) (From Kaufmann and Scheffen (1992), with permission; based on photographs by Boyd and Hamilton (1970))

with a clearly defined maternal component, and the two can be cleanly separated at delivery. Such a separation cannot be achieved in the invasive form of human placentation. This point becomes important as soon as morphologically inexperienced biochemists, endocrinologists, and others isolate respective parts of the organ, then place trust in their putative and designated origin, and draw functional conclusions.

- A typical example is that of the basal plate, often erroneously referred to as "maternal placenta." It is not exclusively composed of maternal cells but rather represents a colorful mixture of trophoblastic (fetal) and endometrium-derived (maternal) cells.
- A corresponding warning is necessary regarding the placental bed. It is often thought to represent only the maternal remains of the placental site after separation of the placenta. Trophoblastic streamers deeply invade the endometrium, however, and even penetrate the myometrium. They remain in utero long after delivery and can be found as fetal admixtures in the placental bed.
- The term "fetal placenta" is also inappropriate. With the possible exception of the central parts of the chorionic plate, there are no placental structures for which the pure fetal composition can be ensured. The marginal zone of the chorionic plate contains decidua, and the same is true for parts of the cell islands and septa. Because the latter may be attached to the villous trees, one is never certain that preparations of it are devoid of maternal tissues, even if we disregard maternal blood and fibrinoid deposits that are partly maternal blood clot products.

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Microscopic Survey

3

For the beginner in placental histology and histopathology, paraffin sections of the organ look confusing because they contain not only a broad variety of differently structured villi but also many nonvillous structures as well. The aim of this chapter is to provide an introduction into those basic histological features that leap to the eye when inspecting a paraffin section and thus to provide a quick orientation for the inexperienced. For this purpose, we have selected a collection of quite conventional photographs from routine histological sections, of routine quality. Labeling of the figures is explained in the text. For further reading concerning the various structures, we refer to later chapters. For quantitative data, we refer to Table A.1.

Ideally, the routine histological examination of the human placenta requires vertically oriented sections that cover all the placental structures from the chorionic plate, via the intervillous space, down to the basal plate (Figs. 3.1 and 3.2). Such sections are easily obtained from most second and third trimester placentas, as well as from the rare in situ specimens obtained by hysterectomy in the first trimester (Fig. 3.1). Tissue biopsies from legal terminations of pregnancy are generally not good for survey pictures; usually, the basal plate together with neighboring tissues such as septa, anchoring villi, and cell columns are either absent or difficult to identify because they are destroyed or mixed up among the villi.

3.1 Typical Histological Features of the First Trimester Placenta

Complete and well-preserved survey sections of the first trimester placenta, such as this vertical section of an in situ specimen from the sixth week post menstruation (p.m.) (Fig. 3.1a), cover the following structures: chorionic plate (b), intervillous space surrounding the placental villi (c–f), cell islands (g), and the basal plate (j–m) from which a septum (h) protrudes into the intervillous space; some anchoring villi are connected via cell columns to the septum (h) or to the basal plate (i).

The intervillous space is the diffuse space that is bounded by the chorionic plate on the one side and the basal plate on the other (Fig. 3.1a). Up to the 12th week p.m., the intervillous space is filled with a clear fluid, arising as a filtrate of maternal blood supplemented by secretions from the endometrial glands (see Chap. 5). The fluid passes around the villous trees, cell islands, and septa and drains into openings the uterine veins. In early pregnancy, development of the villous trees is relatively sparse, and the mean width of the intervillous pores (between neighboring villi) is several hundred micrometers.

Histological specimens of the first trimester chorionic plate (Fig. 3.1b) (see Sect. 9.5) are usually devoid of amnion as for most of this period, the amniotic sac has not expanded sufficiently to reach the chorionic plate. Instead, the extraembryonic coelom is still interposed between the two. As it is a regular constituent of most third trimester survey sections, the chorionic plate is discussed in the next part of this chapter.

If the amnion is missing, as it is in this case, the surface of the chorionic plate (toward the fetus) is covered by an inconspicuous, incomplete layer of mesothelium. It covers a thick layer of chorionic mesoderm in which the chorionic branches of the umbilical vessels are embedded. Toward the intervillous space, the surface is formed in the early stages by a layer of syncytiotrophoblast, which as pregnancy advances is replaced by fibrinoid (Fig. 3.2c).

The tree-like arranged placental villi arise from the chorionic plate and are suspended in the intervillous space, the villous trophoblastic surface being bathed directly by maternal plasma or blood (Fig. 3.1c). The trophoblastic surface of the villi is composed of an outer continuous layer of villous syncytiotrophoblast beneath which is a discontinuous layer of villous cytotrophoblast (Langhans' cells) (see Chap. 6). The villous cytotrophoblast represents the proliferating stem cells for the syncytiotrophoblast that forms the all-important maternal-fetal transport barrier.

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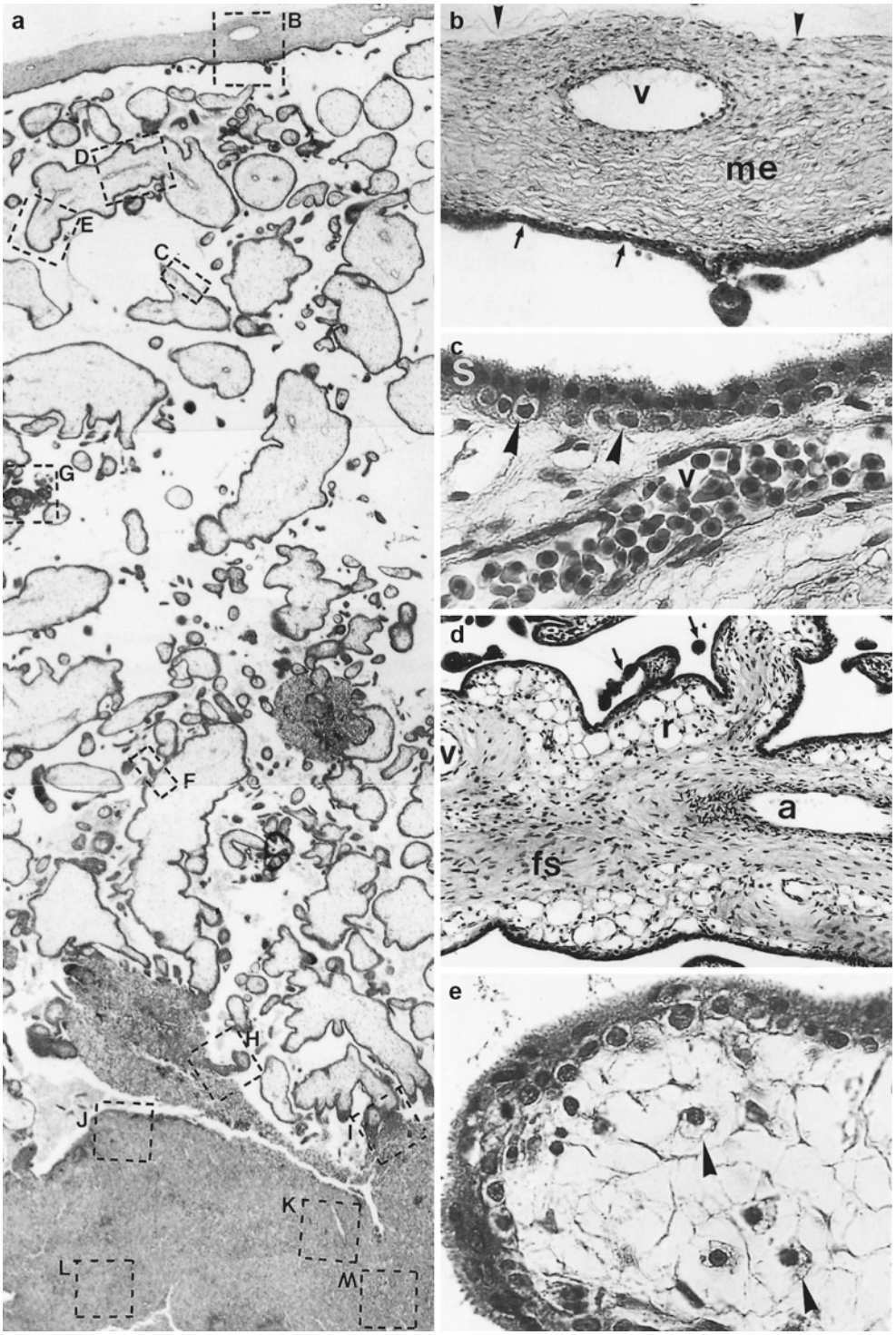

Fig. 3.1 Typical features of the first trimester placenta as seen in paraffin sections following H&E staining. All specimens are from the sixth week p.m., except when otherwise stated. For details, see the text. (**a**) Vertical survey section of an in situ specimen, sixth week p.m. The marked frames refer to the following detailed pictures ×20. (**b**) Chorionic plate. *v* vein, *arrowheads* mesothelium, *me* chorionic mesoderm, *arrows* incomplete layer of syncytiotrophoblast ×100. (**c**) Surface of an immature intermediate villus with trophoblast and a fetal vessel (*v*) containing nucleated red blood cells. *s* syncytiotrophoblast, *arrowheads* cytotrophoblast ×400. (**d**) Transitional form between an immature intermediate villus and a stem villus (18th week p.m.). *a* artery, *v* vein, *r* reticular stroma, *fs* fibrous stroma, *arrows* sprouts ×100. (**e**) Immature intermediate villus showing characteristic reticular stroma with macrophages (*arrowheads*) ×400. (**f**) Mesenchymal villus (*m*) arising from an immature intermediate villus (*i*) and extending into syncytial sprouts (*ss*) ×400. (**g**) Cell island (*ce*) attached to some villi ×100. (**h**) Placental septum (*ps*) connected to a villus (*av*) by a cell column (*cc*) ×200. (**i**) Anchoring villus (*av*) connected to the basal plate by a cell column (*cc*) ×200. (**j**) Surface of the basal plate showing extravillous trophoblast cells (*arrowheads*) embedded in fibrinoid (tenth week p.m.) ×100. (**k**) Deep part of the basal plate showing a uteroplacental vein (*uv*) surrounded by extravillous cytotrophoblast (*ec*) and decidua (*dc*) (37th week p.m., similar to the first trimester situation) ×140. (**l**) Multiple cross sections across a spiral artery (*sa*), the wall of which is replaced by fibrinoid (*arrowheads*) ×100. (**m**) Endometrial glands (*eg*) of the junctional zone embedded in endometrial stroma (*es*) ×200. For further details, see the text

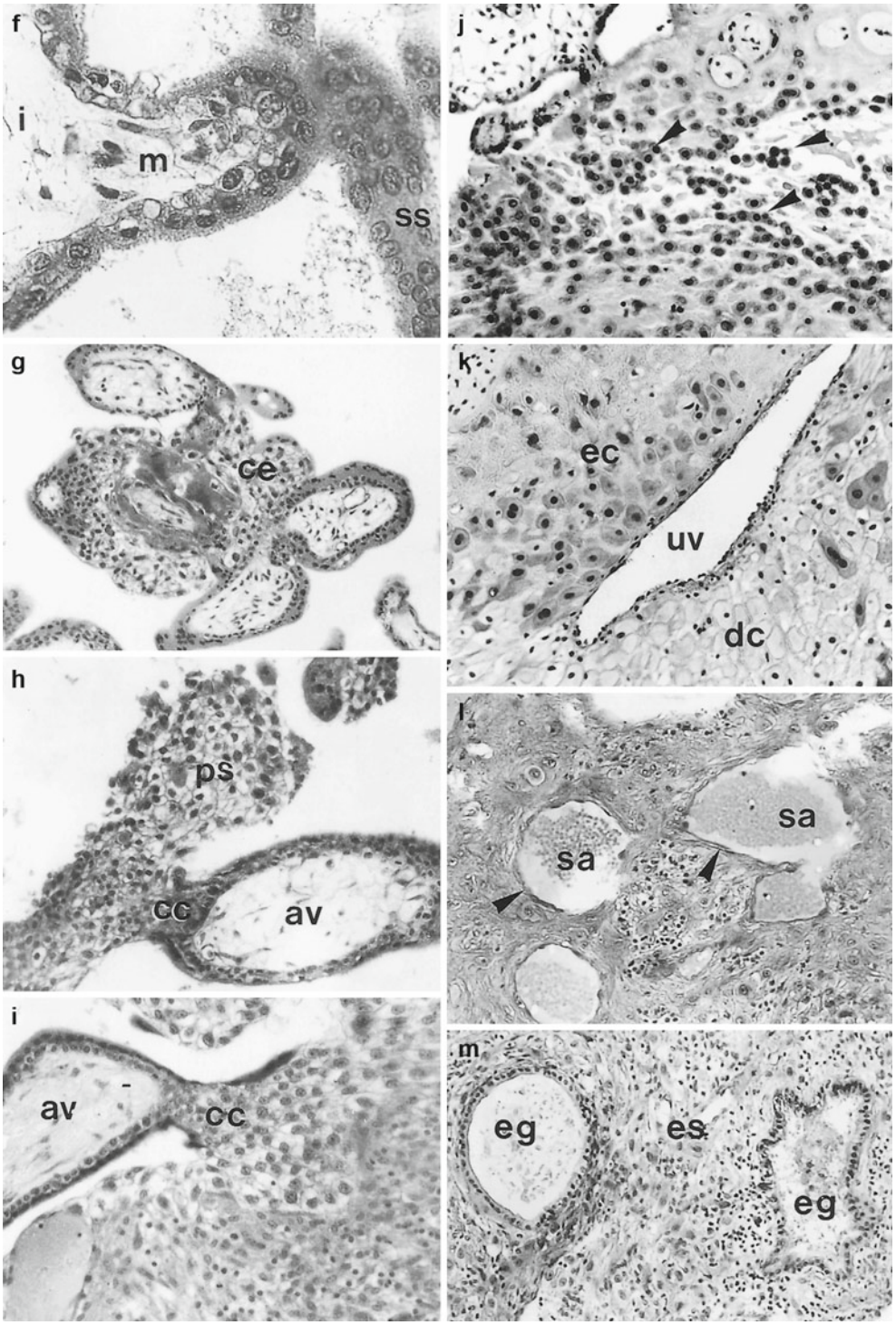

Fig. 3.1 (continued)

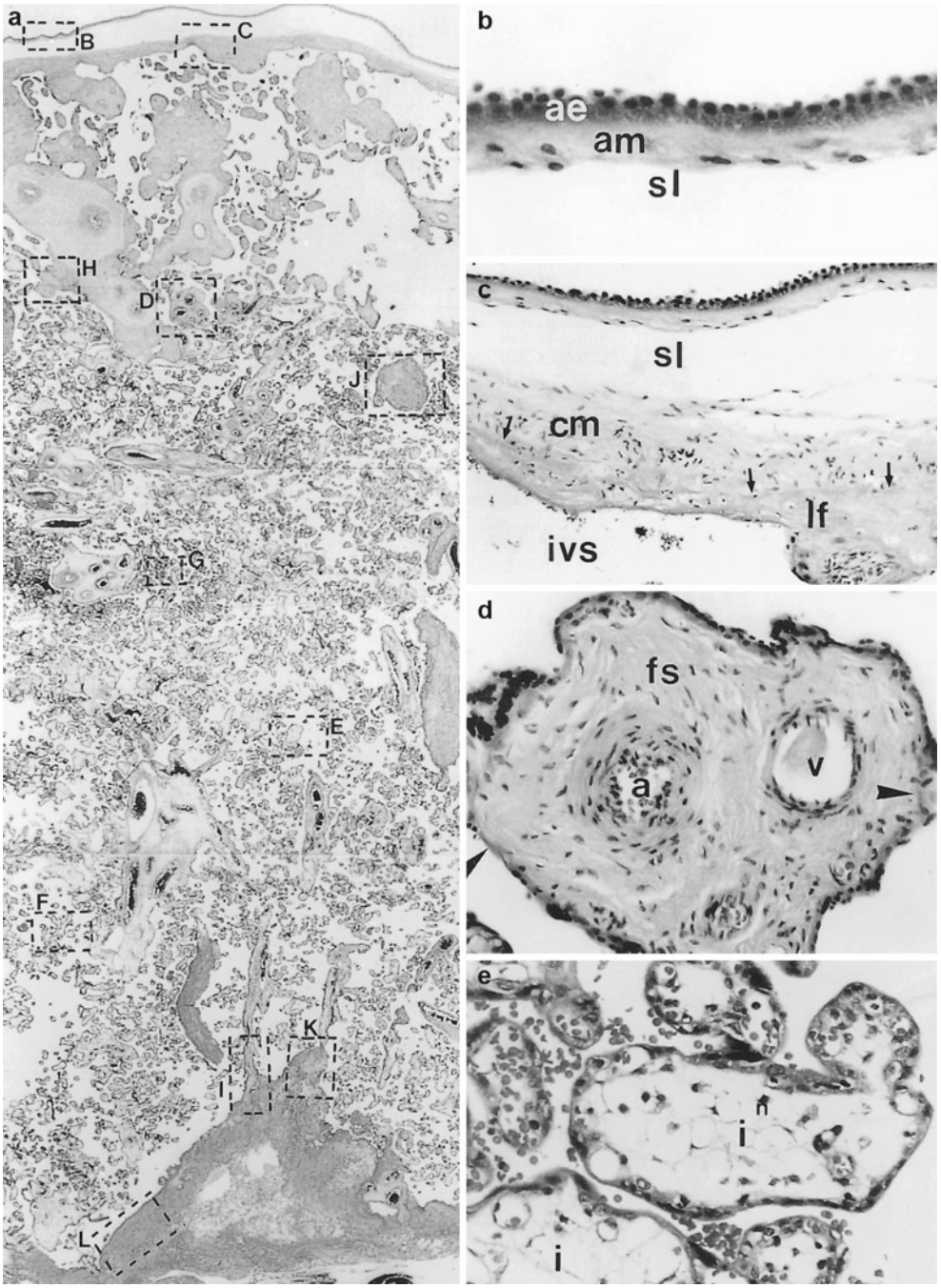

Fig. 3.2 Typical features of the third trimester placenta as seen in paraffin sections following H&E staining. All specimens are from the 40th week p.m. For details, see the text. (**a**) Vertical survey section. The marked frames refer to the following detailed pictures ×10. (**b**) Amnion. *ae* amnionic epithelium, *am* amnionic mesoderm, *sl* spongy layer ×120. (**c**) Chorionic plate, covered by the amnion. *lf* Langhans' fibrinoid stria, *arrows* basement membrane, *cm* chorionic mesoderm, *ivs* intervillous space ×60. (**d**) Peripheral stem villus. *fs* fibrous stroma, *a* artery, *v* vein, *arrowheads* syncytiotrophoblast ×180. (**e**) Two immature intermediate villi (*i*) surrounded by some mature intermediate and terminal villi ×180. (**f**) A longitudinally sectioned mature intermediate villus (*mv*) together with some terminal villi and some villous fibrinoid necrosis (*if*) ×180. (**g**) A group of terminal villi (*t*) showing considerable syncytial knotting (*k*) ×360. (**h**) A small stem villus, the trophoblastic cover of which is partly replaced by a thick plug of perivillous fibrinoid (*f*) ×180. (**i**) An anchoring villus (*av*), connected to the basal plate by fibrinoid (*rf*) as the originally connecting cell column has vanished ×180. (**j**) Cell island. *mf* matrix-type fibrinoid ×90. (**k**) Tip of a placental septum ×90. (**l**) Basal plate with obvious layering. *rf* Rohr's fibrinoid, *nf* Nitabuch fibrinoid, *dc* decidual cells, *ec* extravillous cytotrophoblast, *v* vein ×90. For further details, see the text

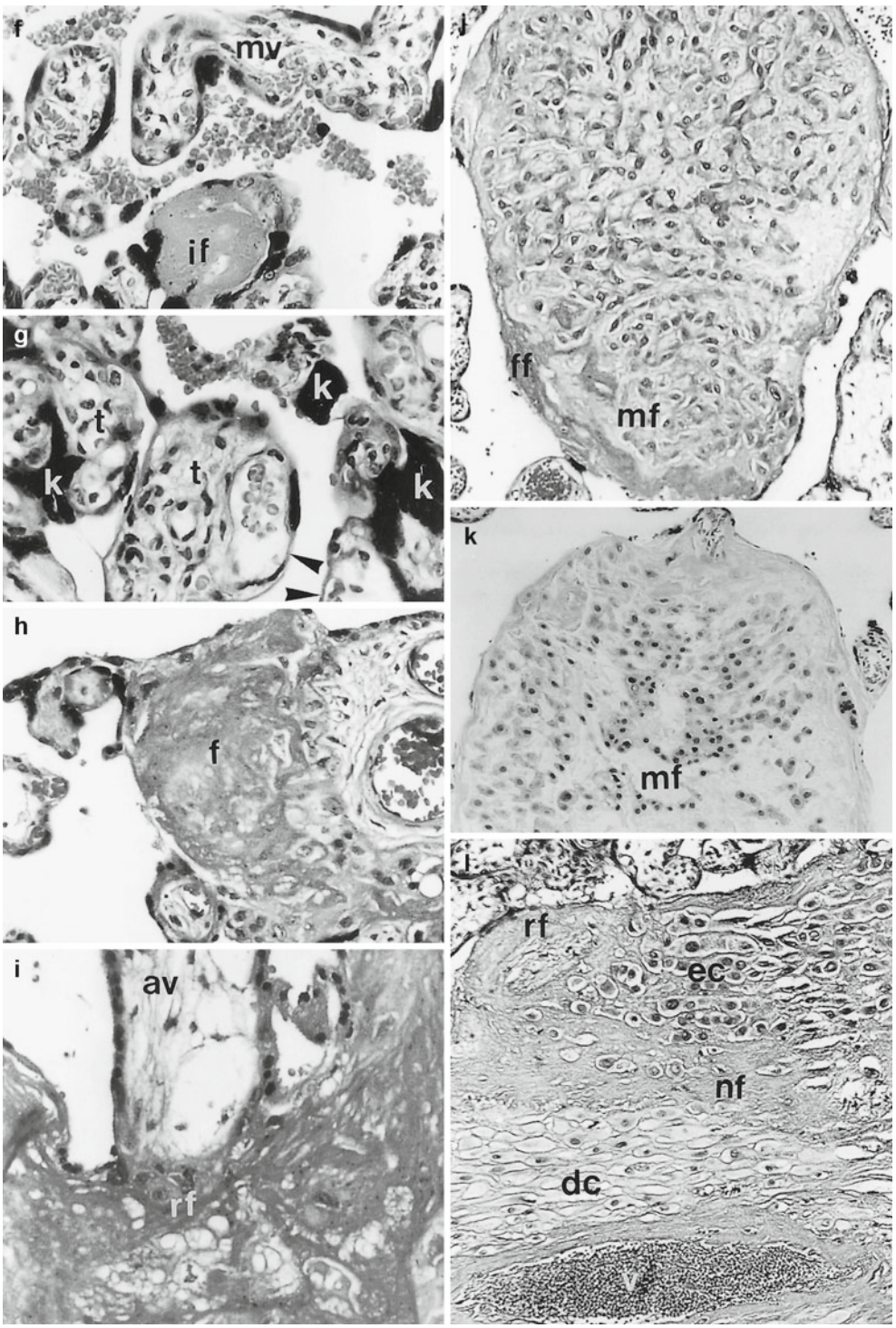

Fig. 3.2 (continued)

The stroma of the villi is composed of the fetal vessels, which are embedded in a mixture of fixed connective tissue cells, macrophages, and connective tissue fibers. Throughout the first 2 months of pregnancy, nucleated red blood cells are a regular finding inside the fetal villous vessels (see Sect. 12.8). These offer a high resistance to flow, and so an effective fetal circulation to the placenta is not established until toward the end of the first trimester.

Very early in gestation, the central stems of the villous trees show signs of fibrosis around larger central vessels that gradually acquire the structural characteristics of arteries and veins (Fig. 3.1d). The superficial stromal layer continues to have an unfibrosed, reticular appearance (see Sect. 7.1.1). Before the eighth week p.m., even the largest villi show a homogeneous mesenchymal stroma; arteries and veins are also absent.

Most of the large, bulbous villi of the first and second trimesters are the immature intermediate villi with a reticular stroma and little fibrosis (Fig. 3.1e). The fixed connective tissue cells surround channel-like spaces within the reticular stroma (Fig. 3.1j; see Sect. 7.1.2). These channels contain villous macrophages (Hofbauer cells) that are easily identifiable by their rounded shape and their vacuolated or granular cytoplasm (see Sect. 6.4).

The peripheral branches of the immature intermediate villi are the mesenchymal villi (see Sect. 7.1.5). They usually have diameters of 60–200 mm, are characterized by a dense cellular stroma, and do not possess many fetal vessels or collagen fibers (Fig. 3.1f). Peripherally, the mesenchymal villi extend into syncytial sprouts, representing multinucleated syncytiotrophoblastic structures. Very often, the sprouts appear as dark cross-sectional profiles (Fig. 3.1d) that seemingly do not connect with villous surfaces. As some of these sprouts break off and are deported into the maternal circulation, it is necessary to trace them through serial sections to confirm whether they are attached or not.

Syncytial or trophoblastic sprouts are the first steps in the formation of new villi. They are invaded by mesenchymal stroma and by fetal capillary sprouts and thus transformed into mesenchymal villi. Later, the mesenchymal villi grow in size and achieve the typical structure of immature intermediate villi (see Sect. 7.2.2). For a description of the structural aspects of syncytial sprouting and knotting, see Chap. 6 (Sect. 6.1.9); for their histopathological significance, see Sect. 15.2 and Chap. 14.

Cell islands are accumulations of extravillous trophoblast cells at the tips of floating villi (see Sect. 9.8.8). They are directly continuous with villous cytotrophoblast in places where the villous syncytiotrophoblast is interrupted (Fig. 3.1g). Parts of the surfaces of these islands may be covered with plaques of syncytiotrophoblast. The extravillous trophoblast cells are usually embedded in matrix-type fibrinoid (see following and Sect. 9.3). Cell islands most likely represent the proximal parts of anchoring villi that have become separated from the basal plate.

Placental septa are veil-like or pillar-shaped extensions of the basal plate that protrude into the intervillous space (Fig. 3.1h). They are rudimentary walls that fail to completely subdivide the intervillous space into separate chambers. Structurally, they show the same composition as the basal plate, as they contain mostly extravillous trophoblast cells and sometimes also some decidual cells. Very often, anchoring villi can be seen attached to the septa. At this stage of pregnancy, they belong to the mesenchymal or immature intermediate type of villi. Cross sections of the tips of septa look like cell islands and can be confused with the latter. For further information, see Chap. 9 (Sects. 9.8.7 and 9.8.8).

Anchoring villi are villi that are connected to the basal plate or placental septa (see Fig. 3.1h, i) via cell columns. They stabilize the position of the villous trees in the maternal intervillous blood stream. For development, see Chap. 5 (Sect. 5.4). Cell columns are the trophoblastic feet of the anchoring villi. In early stages of pregnancy, they consist of several layers of proliferating extravillous cytotrophoblast serving as a proliferative source of villous as well as of basal plate cytotrophoblast (see Sect. 9.1).

The basal plate (Fig. 3.1j) is the boundary of the intervillous space and represents that part of the maternal-fetal junctional zone that adheres to the delivered placenta (see Sect. 9.7). The basal portion of the maternal-fetal junctional zone, that which adheres to the myometrium and remains in utero after delivery, is called the placental bed. The basal plate is composed of an admixture of extravillous trophoblast cells, various endometrial stromal cells, decidual cells, uteroplacental vessels, maternal endothelial cells, and endometrial glands, all embedded in ample fibrinoid that makes up the glossy to fibrillar ground substance.

Extravillous trophoblast cells (Fig. 3.1k) is the generic term for all trophoblast cells located outside the villi: in the chorionic plate, cell islands, septa, basal plate, cell columns, and membranes. Apparent inhomogeneities within the population are mostly the result of different stages of proliferation and differentiation: Those cells resting on the basal lamina facing the chorionic mesoderm (chorionic plate, membranes) or the villous stroma (cell columns, cell islands) are the proliferating stem cells (Langhans' cells), whereas the nonproliferating extravillous trophoblast cells, which have lost contact to the basal lamina, represent more highly differentiated, invasive daughter cells of the former (see Sect. 9.1). Histologically, extravillous trophoblast cells appear as rounded to polygonal or spindle-shaped cells that may be isolated or grouped in strings; they differ from decidual cells in that, in paraffin sections, most of the cell bodies show nuclear cross sections (see Figs. 3.1k and 3.2l).

Decidual cells are enlarged endometrial stromal cells that have elongated, partly branched bodies (Fig. 3.1k) surrounded by a prominent basement membrane. Neighboring decidual cells are usually arranged in parallel, resulting in a peculiar histological appearance: All the cellular sections in one area have the same shape, which is either round, ellipsoid, or longitudinal (Fig. 3.2l), depending on the sectional angle. Because the cell body is elongated, cross sections of the ovoid nuclei are rarely found (see Sect. 9.2). Uteroplacental veins are endothelial tubes, surrounded by few regressive medial and adventitial cells, embedded in decidua and extravillous trophoblast cells; the latter rarely invade the venous walls and never the venous lumina (see Sect. 9.8).

Uteroplacental arteries (spiral arteries) arise from the arcuate arteries running circumferentially around the uterus, and penetrate the endometrium (Fig. 3.1l). Because of their shape, usually several cross sections are found close together (see Sect. 9.8). During pregnancy, the walls of the arteries undergo extensive remodeling, referred to as physiological conversion, under the influence of extravillous trophoblast cells. In the first trimester, a subset of these trophoblast cells migrate down the lumens of the arteries, forming large plugs that occlude the mouths of the arteries and prevent maternal blood from entering the intervillous space. These plugs become dislocated at the start of the second trimester, allowing onset of the maternal arterial circulation.

Beneath the basal plate, the endometrial glands can be found (Fig. 3.1m). These are highly active throughout the first 2 months of pregnancy, forming round to star-shaped lumina lined by columnar epithelium. During much of the first trimester, the decidua basalis beneath the placenta has a thickness of 5–6 mm. The gland secretions are discharged into the intervillous space through openings in the developing basal plate (see Sect. 5.5). The glands are surrounded by endometrial stroma or decidualized endometrial stromal cells, extravillous trophoblast cells, and maternal uterine natural killer cells. In subsequent stages of pregnancy, the glands regress, and only epithelial remainders may be found (see Sect. 9.2).

3.2 Typical Histological Features of the Third Trimester Placenta

Third trimester sections of the placenta, such as this vertical survey section of a mature placenta (Fig. 3.2a) shows, are more difficult to examine as the villi are smaller and often closely juxtaposed so that it is difficult to identify the intervillous space in between. This section covers the chorionic plate (c), including the amnion (b), different types of villi (d–g), fibrinoid deposits in various locations (c, f, h–l), anchoring villi with rudimentary cell columns (i), cell islands (j), septa (k), and the basal plate (l).

In contrast to the first trimester situation, the width of the intervillous space is highly variable with large "subchorionic lakes" below the chorionic plate and narrow intervillous pores between the terminal villi (Fig. 3.2a). These pores are of capillary dimensions only. A generally much wider intervillous space indicates deficiency of terminal villi as in persisting villous immaturity (e.g., Rhesus incompatibility) and in early-onset IUGR (see Chap. 15). A generally much tighter intervillous space is usually correlated with increased numbers of highly branched terminal villi as, e.g., in cases of excessive branching angiogenesis (e.g., late-onset IUGR, often combined with pre-eclampsia) (see Chap. 15). However, these impressions are heavily influenced by the mode of delivery and subsequent leakage of maternal blood from the intervillous space.

The amnion covers the chorionic plate toward the amniotic cavity (Fig. 3.2b). It consists of a single layer of cuboidal to columnar cells that participate in the turnover of the amniotic fluid (see Sect. 11.1.2). Seemingly multilayered segments of the amnionic epithelium represent oblique or tangential sections across the surface in most cases. In addition, nearly 50% of mature placentas also possess foci of real squamous metaplasia of the amnionic epithelium that may become up to 15 cellular layers in thickness (see Sect. 11.1.2). Underneath the amnionic epithelium is a thin layer of amnionic mesoderm (about 15–30 mm in thickness). It is only loosely connected with the next layer, the chorionic mesoderm, via the spongy or intermediate layer, a reticular zone showing larger clefts (Fig. 3.2c). Because of unstable connection, the amnion may start gliding or may even become lost during preparation.

The third trimester chorionic plate is a multilayered structure (Fig. 3.2c). It consists of the spongy layer with numerous clefts, followed by the compact layer of chorionic mesoderm that is separated from the Langhans' fibrinoid stria by a rudimentary basement membrane. On the lower side of this basement membrane, highly variable amounts of extravillous cytotrophoblast can be found. During early pregnancy, they form a complete and usually multilayered stratum; in later pregnancy, this layer becomes rarefied. Some of the cells deeply invade the fibrinoid (see Chap. 11: *Chorionic Plate*) (see Sect. 9.5). Attached to or embedded into the fibrinoid, one finds numerous stem villi, representing the first branches of stem villi branching off from the chorionic plate nearby (see upper third of Fig. 3.2a). The chorionic plate represents the cover of the intervillous space, which lies directly below.

The villous trees measure 1–4 cm in diameter (Fig. 3.2d). Their central branches are made up of stem villi (see Fig. 7.18). These are the large caliber villi that range from 80 to several thousand micrometers in diameter (see Sect. 7.1.1). The highest concentration of stem villi and the largest calibers are found near the chorionic plate. Histologically, they

are characterized by one or several arteries and veins or arterioles and venules with clearly visible muscular walls and are surrounded by a fibrous stroma that contains few paravascular capillaries (see Sect. 7.3.3). Near term, the trophoblastic cover is focally or largely replaced by fibrinoid (see Sect. 6.6.1).

The immature forerunners of the stem villi are the immature intermediate villi (Fig. 3.2e). They are easily identifiable by their large caliber and their pale staining. Larger arteries and veins are usually absent, as are larger amounts of collagen fibers. The prevailing structure within the stroma is a reticularly arranged loose connective tissue with few fetal vessels. It is composed of a net-like arrangement of fixed connective tissue cells that surround round spaces, the so-called stromal channels (see Sect. 7.1.2), which contain the villous macrophages. The latter are characterized by their rounded cell body and their numerous vacuoles or lysosomes.

Immature intermediate villi are the dominant villous type in early pregnancy (Fig. 3.1d, e). At term, they usually persist in small groups (<10% of the total villous volume) in the centers of the villous trees. They are absent in hypermature placentas and their number is increased with persisting immaturity of the placenta (see Sect. 15.4.2).

Mature intermediate villi are slender, multiply curved branches of stem villi that exhibit diameters ranging from 60 to about 100 mm (Fig. 3.2f). They differ from stem villi by the absence of both stromal fibrosis and fetal stem vessels (arterioles and venules) with a media identifiable by light microscopy. Their stroma is composed of slender fetal capillaries embedded into a loose connective tissue that is rich in cells but poor in fibers (see Sect. 7.1.3). Longitudinal sections of mature intermediate villi are easily identifiable, whereas cross sections can easily be confused with terminal villi (see following).

Terminal villi are the grape-like terminal side branches of the mature intermediate villi (see Fig. 7.7 and Sect. 7.1.4). Their diameters range from 40 to about 80 mm (Fig. 3.2g). The dominating structures within the loose stroma are sinusoidally dilated and highly coiled fetal capillaries (see Sect. 7.3.5). Typically, they bulge against the trophoblastic surface and transform this into extremely thin vasculosyncytial membranes that are devoid of nuclei.

The terminal villi, together with mature intermediate villi, represent the main exchange area of the third trimester placenta. Mature intermediate villi with slender capillaries and terminal villi with dilated sinusoids are easily discernible in placentas following early cord clamping and immediate fixation. Placentas that were fixed late after delivery and have lost larger amounts of fetal blood show the collapse of sinusoids so that the terminal villi can no longer be discriminated from mature intermediate villi, which have slender capillaries.

A typical cross-sectional feature of terminal villi, and to a certain degree also of other villous types, is syncytial knotting (Tenney-Parker changes). This term is applied to aggregations of syncytial nuclei that form gentle elevations from the villous surface and may contribute to the formation of trophoblastic bridges connecting neighboring villi. Only a small percentage of respective structures represent real trophoblast protrusions and real bridges in the third trimester (see Sects. 6.1.9 and 15.2). Rather, the vast majority are tangential sections across trophoblastic surfaces of irregularly shaped and branched villi. Toward term, the outer shape of terminal villi becomes more irregular, thus increasing the chance of tangential sectioning.

Fibrinoid is an acellular, intensely staining, eosinophilic material that is mostly related to the intervillous space (Fig. 3.2c, d, f, h). When it replaces the trophoblastic cover of villi, as shown in this figure, it is called perivillous fibrinoid. In other villi, it may replace the stroma beneath a largely intact trophoblastic surface (intravillous fibrinoid, villous fibrinoid necrosis; Fig. 3.2f). Other sites of fibrinoid deposition are a prominent fibrinoid layer below the chorionic plate (Langhans' fibrinoid, Fig. 3.2c), a corresponding layer at the surface of the basal plate (Rohr's fibrinoid, Fig. 3.2i, l), the Nitabuch fibrinoid in the depth of the maternal-fetal junctional zone (Fig. 3.2l), and the extracellular matrix of cell islands (Fig. 3.2j) and septa (Fig. 3.2k).

Despite the fact that fibrinoid appears to be homogeneous histologically, it is composed of two completely different materials: (1) fibrin-type fibrinoid, a blood clotting product that is free of extravillous trophoblast cells and usually in contact with the intervillous space and (2) matrix-type fibrinoid, in which is embedded varying numbers of extravillous trophoblast cells and is itself a secretory product of these cells. Both substances may be deposited close together or separately (see Sect. 9.3).

With the increasing development of free-floating villi that takes place in the course of the last trimester, anchoring villi become less prominent (Fig. 3.2i). They are still connected to the basal plate or to septa by cell columns, but these are much shorter than in early pregnancy (Fig. 3.1h, i). As the proliferative activity of the cytotrophoblast within a column slows down, the cells become rarefied to one layer, and sometimes even that is incomplete. In some cases, extravillous trophoblast may be completely replaced by matrix-type fibrinoid so that the stroma of the anchoring villus directly borders Rohr's fibrinoid of the basal plate. Some cell columns maintain their proliferative activity and thus act as growth zones for the anchoring villi as well as for the basal plate, even near term (see Sect. 9.1).

Cell islands increase in size throughout pregnancy by means of the continuous proliferation of their extravillous trophoblast cells (Fig. 3.2j). Also, the amount of fibrinoid

embedding these cells increases steadily by apposition of fibrin-type fibrinoid from the intervillous space and by secretion of matrix-type fibrinoid from the trophoblast cells. Large cell islands may contain central cavities, or "cysts." They must be considered the result of degeneration of trophoblast cells and subsequent liquefaction. Occasionally, cell islands may contain some decidual cells in addition to extravillous trophoblast cells. Such islands can be interpreted either as cross sections of placental septa or as disrupted parts of anchoring villi. For an understanding of their development, see Chap. 5 (Sect. 5.4).

Placental septa are attached to the basal plate at their base (Fig. 3.2a, k) and are usually composed of extravillous trophoblast and decidual cells, both embedded in much fibrinoid. The septal tips are composed mostly of extravillous trophoblast and matrix-type fibrinoid and are surrounded by fibrin-type fibrinoid. Their cross sections are hardly distinguishable from cell islands.

Only in a few exceptional places is the basal plate of the term placenta as clearly layered as depicted in Fig. 3.2l, showing a superficial stria of Rohr's fibrinoid, followed by extravillous cytotrophoblast, a layer of Nitabuch fibrinoid, and a compact decidual layer. In the latter, a uteroplacental vein is embedded. In most cases, however, there is no clearly defined fetomaternal border but rather extravillous cytotrophoblast, decidual cells, uteroplacental vessels, maternal endothelial cells, and glandular residues intermingled with ample fibrinoid and having no identifiable order (see Sect. 9.7). In such cases, immunohistochemical markers may facilitate orientation.

Placental Types

4

All viviparous vertebrates develop a system of extraembryonic membranes that surround the fetus. The apposition or fusion of these fetal membranes with the uterine mucosa, for purposes of maternal-fetal physiological exchange, initiates the formation of the placenta. The fetus is connected to the membranes by the umbilical cord. The maternal-fetal contact zone, provided by the membranes and the endometrium, represents the placenta.

This nonspecific definition points to the great structural and functional variability of the organ. There is probably no other organ that exhibits such a large degree of interspecies variation. This may reflect the fact that the placenta is a very recent organ in terms of evolutionary history, relative to, for example, the kidney or liver. As such, it is still evolving. Only one structural component is common to all placental types, namely, the existence of two separate circulatory systems: the maternal and fetal placental circulations. Under normal conditions, the vessels of both systems remain separated by several tissue layers throughout pregnancy. Even the origin of these separating tissue layers, making up the so-called placental barrier or interhemal membrane, varies. These may be derived from fetal tissues, including (1) the blastocyst wall (trophoblast), which following fusion with the fetal mesenchyme is called the chorion; (2) the allantois, which is an extracorporeal extension of the embryonic urinary bladder; or (3) the yolk sac, an extracorporeal vesicular extension of the gut (Fig. 4.1). These membranes are complemented by the amnion, which forms a fetally derived membranous sac surrounding the embryo and maintains it in an aqueous environment. In some cases, these fetal constituents are complemented by derivatives of the maternal endometrium.

The placenta is unique among all other organs in that it conducts the functional activities of most fetal organs (except the locomotor apparatus and the central nervous system) from its early beginning on throughout its development. The following fetal functions are partially or completely accomplished by the placenta during pregnancy as a substitute for still immature embryonic and fetal organs:

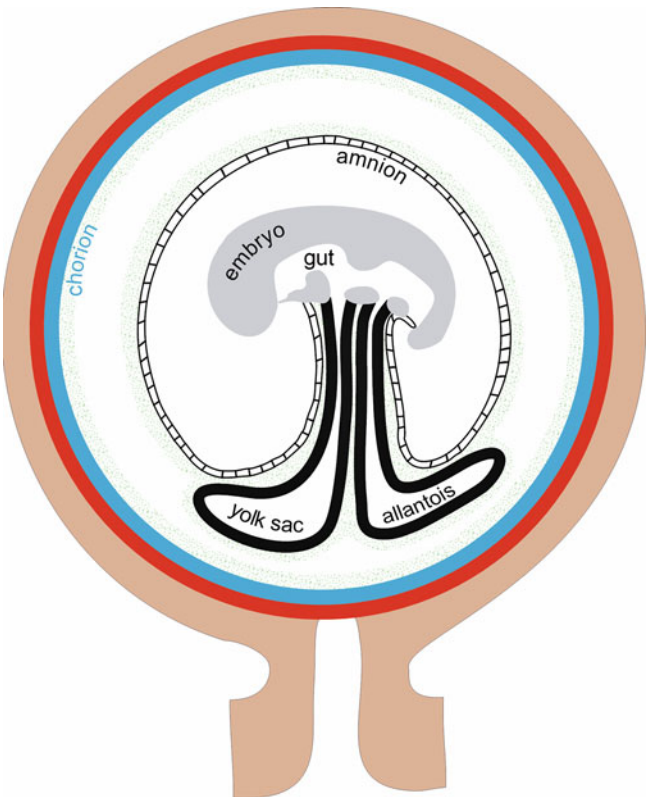

Fig. 4.1 Synoptic representation of the fetal membranes (*black*, *blue*) that may contribute to the formation of a placenta. Maternal tissues are colored *red* and *brown*. The trophoblast (*blue*), as a derivative of the blastocyst wall, together with the fetal mesenchyme (*gray*, *dotted*) forms the chorion, which is the main exchange membrane of most mammals. The chorion does not develop its own vessels but, rather, becomes vascularized either by the allantois (*black*) (→ chorioallantoic placenta) or by the yolk sac (*black*) (→ choriovitelline placenta). In some species, the chorion is replaced locally by the yolk sac (*black*) (→ yolk sac placenta). The amnion (*black*) is unvascularized and never replaces the chorion; rather, it serves as an additional, inner membrane that separates the chorion or yolk sac from the amniotic fluid

1. Gas transfer (later the function of the lung)
2. Excretory functions, water balance, pH regulation (later the function of the kidney)

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3. Catabolic and absorptive functions (later the function of the gut)
4. Synthetic and secretory functions of most endocrine glands
5. Numerous metabolic and secretory functions of the liver
6. Hematopoiesis of the bone marrow (during early stages of pregnancy)
7. Heat transfer of the skin
8. Immunological interactions and protection

It is very unlikely that these numerous functional requirements can be fulfilled by the phylogenetic development of an identical structural solution for all species, as species exhibit tremendous variation in body size, length of pregnancy, litter size, and living conditions. Instead, there has been the development of numerous placental types, differing from each other with respect to their overall shape, the nature of the maternal-fetal interdigitation and structure of the maternal-fetal barrier, and maternal-fetal blood flow interrelationships.

Placentas can be classified into two fundamental categories depending on the source of the blood vessels which vascularize the chorion: choriovitelline if the vessels come from the yolk sac and chorioallantoic if they come from the allantois (Fig. 4.1). Of the two types, the choriovitelline placenta is the phylogenetically older and represents the definitive placenta of most marsupials. In eutherian mammals, a choriovitelline placenta often functions transiently in early pregnancy, but the later definitive placenta is always of the chorioallantoic type. In some, such as the rodents, both types of placenta may operate in parallel, performing separate functions. Here, we present a brief overview of the principal types of chorioallantoic placenta, for comparison with that of the human. For more detailed information, we refer to the monographs on comparative placentology by Steven (1975), Ramsey (1982), Mossman (1987), and Wooding and Burton (2008) and to the reviews by Enders and Carter (2006), Carter (2007), Enders (2009), and Freyer and Renfree (2009).

Among the numerous possibilities for classifying chorioallantoic placentas (Steven 1975; King 1982; Ludwig 1981; Ramsey 1982; Mossman 1987; Dantzer et al. 1988), the following characteristics have proved to be the most successful for characterizing the organ structurally and, to a limited degree, functionally:

1. Overall shape and surface extension around the chorionic sac
2. Nature and intensity of interdigitation of the maternal and fetal tissues
3. Number, kind, and structure of the tissue layers separating maternal and fetal blood
4. Spatial arrangement of maternal and fetal vessels or of blood flow directions, with respect to one another

It is impossible to cite here the vast literature comparing the various placental types. Rather, for each placental type, or for each group of animals, we refer to one or two detailed publications that may serve as characteristic illustrations.

4.1 Placental Shapes

The capacity for maternal-fetal and fetal-maternal transfer depends largely on the contact area available for exchange. The contact area of the simple chorionic sac (the derivative of the blastocyst wall and outer layer of the fetal membranes) with the endometrium appears insufficient to meet fetal demands, even if it involves the entire surface, for this placental type does not exist in mammals. Rather, all mammals increase the exchange area by interdigitation of the opposing maternal and fetal tissue surfaces. In most cases, the surface enlargement by interdigitation is so intense that enough exchange surface can be provided even if interdigitation takes place only on a limited surface area of the chorionic sac. This interdigitating part is called the placenta, whereas those parts of the chorionic sac that maintain a more or less planar apposition to the uterine surface are called the smooth chorion, chorion laeve, paraplacenta, or simply the outer fetal membranes. Each order or suborder of mammals has a typical shape and surface extension of its placenta.

In comparative placentology, the following basic placental shapes have been defined, although it should be recognized that intermediate shapes do occur in some species:

- Diffuse placenta (placenta membranacea). Maternal-fetal interdigitations extend over the entire surface of the chorionic sac with no obvious gross morphological features (Fig. 4.2a). This type is seen in Perissodactyla (Allen and Wilsher 2009), Cetacea (Wislocki and Enders 1941), Suidae (MacDonald and Bosma 1985), Tylopoda (Ramsey 1982), and some lower primates (Ramsey 1982).
- Cotyledonary placenta. In these placentas, the maternal-fetal interdigitations are restricted to small circular or elliptical patches over the surface of an elongated chorionic sac, such as those in ruminants (King et al. 1979) (Fig. 4.2b). The villi only form opposite specialized nonglandular areas of the endometrium referred to as caruncles, the number and size of which are species specific.
- Zonary placenta. In carnivores (Miglino et al. 2006), Sirenia (Carter et al. 2008), Hyracoidea (Mossman 1987), and Proboscidea (Wooding et al. 2005), the placenta forms an equatorial ring-like zone over the chorionic sac, surrounding the fetus in a girdle-like fashion (Fig. 4.2c).
- Discoid placenta. In this type, the interactions are restricted to one or more poles of a spherical chorionic sac. Several lower and higher primates (Luckett 1970; Ramsey 1982), as well as the *Tupaia* (Kaufmann et al. 1985; Luckhardt et al. 1985), develop two disk-like areas of intimate contact, a double discoid placenta. In rodents

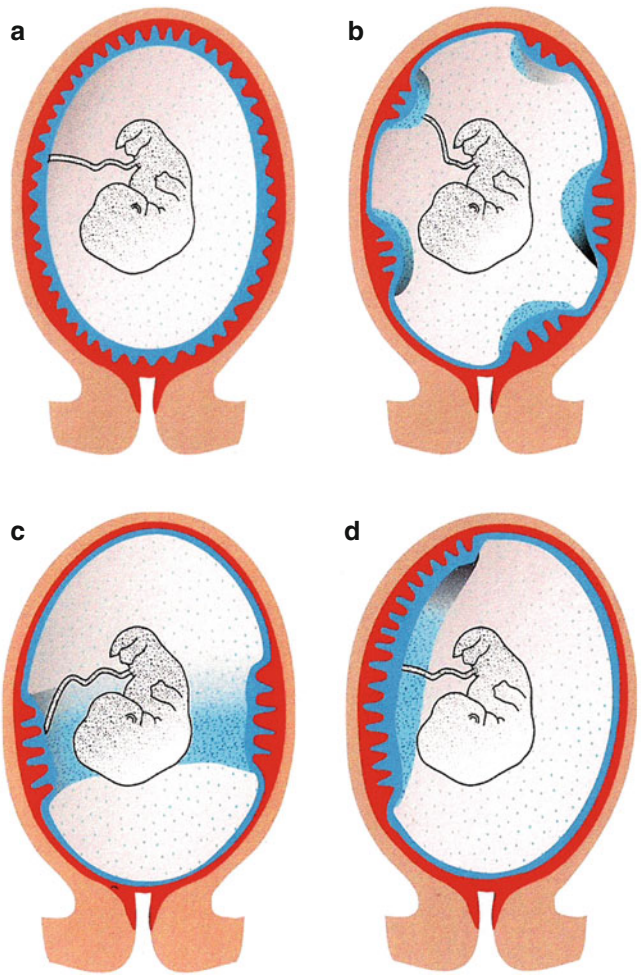

Fig. 4.2 All known placental types show interdigitations between the maternal (*red*) and fetal (*blue*) components. Depending on the extent of these interdigitations, several placental shapes are described. (**a**) Diffuse placenta, (**b**) cotyledonary placenta, (**c**) zonary placenta, and (**d**) discoid placenta. For details, see text (*Source*: Modified after Kaufmann (1981), with permission)

(Kaufmann and Davidoff 1977; Georgiades et al. 2002), the great apes (Ludwig and Baur 1971), and the human (Boyd and Hamilton 1970), there is a single discoid placenta (Fig. 4.2d).

Variations in the shape of the human placenta do occur, but these represent disordered development due to implantation at abnormal sites, such as lateral in the uterus, and should not be considered homologues of the situation in other species.

4.2 Types of Maternal-Fetal Interdigitation

If that part of the surface area of the chorionic sac in which maternal-fetal interdigitation takes place is reduced, it must be compensated for by an increased intensity of the interdigitations. This results in five different types of maternal-fetal interdigitation:

- Folded type. The simplest form of interdigitation, which consequently is combined with the large-surfaced diffuse placenta (Fig. 4.3a). It is characterized by poorly branching ridge-like folds of the chorion that fit into corresponding grooves of the uterine mucosa. Examples are seen in the pig (MacDonald and Bosma 1985) and some lower primates (Ramsey 1982) that have a diffuse placenta.
- Lamellar type. A generally similar but more complex construction is typical for the lamellar type of placenta (Fig. 4.3b) found in the carnivores (Leiser and Kohler 1984). In this case, the ridges multiply and branch into complicated systems of slender chorionic lamellae, oriented in parallel to each other and separated by correspondingly branching endometrial folds.
- Villous type. Even more exchange surface per unit placental volume is provided by a tree-like branching pattern of the chorion, resulting in the placental villous tree. The villi fit into corresponding endometrial crypts or are directly surrounded by maternal blood depending on the degree of invasion. A villous type of placenta (Fig. 4.3d) exists in ruminants (Steven 1975; Kaufmann 1981) and in higher primates (King and Mais 1982; Boyd and Hamilton 1970).
- Trabecular type. This type represents an intermediate stage between the lamellar and the villous condition (Fig. 4.3c). It has been described for some platyrrhine monkeys, such as *Callithrix* (Luckett 1974; Merker et al. 1987), and is characterized by branching folds from which leaf-like and finally finger-like villi branch off.
- Labyrinthine type. The most common kind of interface can be found in the labyrinthine type of placenta (Fig. 4.3e). It has been described in rodents (Kaufmann and Davidoff 1977), lagomorphs (Mossman 1987), insectivores (Malassiné and Leiser 1984), bats (Wimsatt and Enders 1980), and some New World monkeys (Ramsey 1982; Kaufmann et al. 1985; Mossman 1987). Characteristically, a tissue block of trophoblast is penetrated by a complex network of channels filled with maternal blood or fetal capillaries.

Recently, a close association has been demonstrated between the degree of interdigitation and gestation length (Capellini et al. 2011). Species with highly interdigitated labyrinthine placentas have gestation lengths 44% of those associated with villous or trabecular placentas. No relationship was found with birth weight or brain size, however. The authors suggest that ecological pressures, such as selection to produce multiple litters in seasonal environments, may have driven the evolution of more efficient placentas. In this scenario, reduced maturation of the brain at birth might be viewed as one of the costs, for altriciality is associated with short gestations.