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Pathologic Evaluation of Normal and Perfused Term Placental Tissue

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ABSTRACT

This study reports for the 1st time the incidence and interobserver variation of morphologic findings in a series of 34 term placentas from pregnancies with normal outcome used for perfusion studies. Histologic evaluation of placental tissue is challenging, especially when it comes to defining ''normal tissue'' versus ''pathologic lesions.'' A scoring system for registration of abnormal morphologic findings was developed. Light microscopic examination was performed independently by 2 pathologists, and interobserver variation was analyzed. Findings in normal and perfused tissue were compared and selected findings were tested against success parameters from the perfusions. Finally, the criteria for frequent lesions with fair to poor interobserver variation in the nonperfused tissue were revised and reanalyzed. In the perfused tissue, the perfusion artefact ''trophoblastic vacuolization,'' which is believed to represent dilated transtrophoblastic channels, was reproducible and significantly correlated to the perfusion marker ''fetal leakage.'' In longer perfusions, microscopy of the perfused cotyledon revealed bacteria in the fetal vessels. This finding led to an adjustment in the perfusion protocol with addition of antibiotics to the medium. In the ''normal'' tissue, certain lesions were very frequent and showed only fair or poor interobserver agreement. Revised minimum criteria for these lesions were defined and found reproducible. This study has emphasized the value of pathologic examination as a supplement in placental perfusion models. Examination of the perfused cotyledon for trophoblastic vacuolization is recommended as an additional quality marker in perfusion models. The study also underlines the need for exact definitions of abnormality in frequent placental lesions.

Key words: diagnostic criteria, frequent lesions, interscorer variation, placental morphologic evaluation, placental perfusion, term placental histology

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INTRODUCTION

The Copenhagen Placental Perfusion Model (CPPM) was established in 2004 to examine and evaluate transport of toxic and nontoxic substrates through the fetomaternal barrier in an in vitro placental tissue model based on fresh donated placentas from uncomplicated term pregnancies [1,2]. The use of histologic evaluation to assess the quality of a placental perfusion has been suggested and is further investigated in this paper. Tissue samples from the perfused cotyledon are often taken for visualization of the study substrate and for detection of changes in expression of placental proteins. The morphology, however, of perfused placental tissue is only briefly described in the literature. On histologic light microscopic examination, dilated fetal vessels and absence of red blood cells are usually noted. Ultrastructurally by electron microscopy, some have reported normal morphology, whereas others have observed distended endoplasmatic reticulum. Sometimes vacuoles in the trophoblast layer of the villi have been observed, especially after hypoxic perfusions. This trophoblastic vacuolization has by electron microscopy studies been attributed to dilatation of pressure-dependent transtrophoblastic channels [3–7].

In this study, the placentas were fixed, and both perfused and nonperfused tissue was examined. The examination of the perfused tissue was an opportunity to investigate the occurrence and interobserver variation of perfusion-related histologic artefacts (perfusional changes). The nonperfused tissue offered a unique chance for examination of ''normal'' placental tissue, which under usual circumstances would not have been sent for pathologic examination. To the best of our knowledge, a prospective series of detailed morphologic examination of perfused placentas has not previously been published.

Morphologic evaluation of the placenta is challenging. The placenta is a temporary organ undergoing extreme changes and explosive growth throughout its short life span. The literature on placental histologic findings is vast but mainly based on and developed for the

examination of placentas sent for gross and histologic examination due to pregnancy- and birth-related complications, such as infection, intrauterine growth retardation, and fetal death. Several guidelines on practical handling of placentas are available in textbooks and articles, as are recommendations for reporting pathologic entities and specific subentities [8–18]. These are constantly being updated in the literature. Placental evaluation is complicated by the fact that well-defined pathologic entities in some cases are causative, explaining a negative pregnancy outcome, while in other cases are incidental findings in uncomplicated pregnancies. Large studies of unselected series of placentas have been published [18–21], but only few papers have reported series of morphologic findings in normal term placentas [22,23]. In these publications, the criteria for reporting specific entities vary and are not always described in precise detail, making comparison between studies difficult. In particular, the difficult threshold between a normal and an abnormal occurrence of a placental component, such as fibrin or calcification, is usually not defined in detail. The reproducibility of pathologic observations is the subject of many studies but is usually examined in the context of specific pathologic entities (eg, inflammation) [24–33]. To the best of our knowledge, interobserver variation for histologic changes in a series of term placentas from uncomplicated pregnancies has not previously been published.

The primary aims of this study were (1) to determine whether in vitro perfusion introduces significant perfusional changes (histologic artefacts) that might be confused with pathologic lesions and (2) to detect reproducible perfusional changes in the tissue, which might be used as morphologic markers of success or failure of perfusion. The secondary aim was to report the occurrence and interobserver agreement for histologic findings in normal and perfused term placental tissue. First, a practical scoring system for morphologic findings in normal (nonperfused) and perfused tissue was developed according to which the findings were quantified and the interobserver variation evaluated. Second, findings in normal and perfused tissue were compared and selected findings were tested against success parameters from the perfusions. Finally, the criteria for frequent lesions with fair to poor interobserver variation in the nonperfused tissue were revised and reanalyzed.

MATERIALS AND METHODS

The normal (nonperfused) tissue was examined by standard gross and light microscopic examination by 2 experienced senior pathologists from different institutions with special interest and training in placental pathology (L.L.M., L.G.L.). A total of 34 placentas from uncomplicated pregnancies used in the CPPM from 2008 to 2010 were included in the study. Inclusion criteria were written informed consent, an uncomplicated pregnancy and uneventful birth by vaginal delivery or elective caesarean section, and the completed perfusion of a single cotyledon.

The 34 placentas (caesarean section [$n = 29$] or vaginal birth [$n = 5$]) consisted of 33 singletons and 1 monochorionic twin placenta. Gestational age (weeks + days) ranged from 37 + 7 to 41 + 3. The births resulted in live children of an average length and weight of 52 cm and 3660 g (range 48–60 cm and 2672–4840 g). The average age of the mother at delivery was 33.8 years (27–44 years).

Placental in vitro perfusion

The ex vivo recirculating placental perfusion model set up in Copenhagen is described in more detail elsewhere [2]. The study protocol for placental perfusions has been approved by the regional Ethics Committee (KF 01-145/03 + KF(11) 260063) and the Data Protection Agency. The placenta is received directly after birth and is injected with Krebs Ringer Buffer supplemented with glucose and heparin and transported to the perfusion laboratory. A single placental unit (fetal cotyledon) is cannulated in fetal vein and artery, and a closed circuit without significant fetomaternal leakage qualifies the unit for insertion in the perfusion chamber. The criterion for successful perfusion is a volume loss (fetal leakage) of <3 mL medium/hour from the fetal reservoir.

Immediately after termination of the perfusion, the perfused fetal cotyledon (distinguishable by the white appearance due to depletion of red blood cells) is isolated, weighed, marked, and stored in 4% formaldehyde until preparation. The unperfused tissue surrounding the cotelydon in the perfusion chamber and any cotelydons unsuccessfully perfused are not analyzed. All the remaining (nonperfused/normal) tissue from the donated placenta, including membranes and umbilical cord, is marked and similarly stored in formaldehyde until pathologic examination.

Pathologic examination

After fixation, the placental disc, umbilical cord, and free membranes were grossly examined and described (by L.L.M.) using standard recommendations for pathologic examination of placentas [9]. Three cross-sections of umbilical cord, 1 membrane roll, 2 sections of representative nonperfused tissue, and 2 sections of representative perfused tissue were submitted for histologic examination as a minimum. Additional sections from any observed focal lesions were submitted from both nonperfused/normal and perfused tissue.

Two original and identical sets of hematoxylin and eosin–stained slides were prepared simultaneously and examined microscopically by the 2 pathologists (L.L.M. and L.G.L.), who were blinded to the results of the perfusions, including success variables. Based on principles described in the updated literature on placental pathology, a diagnostic scoring system was developed. In this system, selected histologic findings including observed in vitro perfusion-induced changes were scored from 0 (not present) to a maximum of 3. When possible, the scoring system was adapted to reflect the recommendations of reporting and grading/staging in the literature [11,13,14,27–30,34–36]. The 1st 6 cases served as pilot

study cases. After detailed discussion of specific entities and interpretation of the findings by the 2 pathologists, the diagnostic criteria were refined and the 1st 6 cases revised according to the consensus reached. The revised scoring system was then applied to the following 28 cases. The cases were initially examined and scored by each of the pathologists separately. Later, each case was evaluated in a joint meeting and any disagreements in reporting were discussed until consensus was reached. On rare occasions (4 in normal and 3 in perfused tissue), no consensus could be reached, and in these circumstances, the average grade was assigned to the finding. Interobserver variation was analyzed for cases 7–34.

Data analysis

When possible, interobserver variability was tested by Cohen's unweighted Kappa (κ) analysis for 2 observers using PASW statistics data editor 18 (SPSS). When all observations in an observational category were 0, the κ value was manually calculated [37]. Kappa values were interpreted as follows: $\kappa \leq 0.20$ poor agreement; 0.21–0.61 fair/moderate agreement; 0.61–1.00 good/near-perfect agreement. On some occasions, κ could not be calculated (denominator = 0). On other occasions, when only 2 observational categories were present and all observations in 1 observational category were 0, the κ value was 0 regardless of the amount of disagreements between the observers. On these occasions, κ was deemed invalid. Based on results from entities with valid κ, an alternative evaluation system based on the amount of observational disagreements was set up. When κ was invalid, the interobserver variation was interpreted as follows: observational disagreements ≥4 poor agreement; 2–3 fair agreement; 0–1 good agreement [30,37].

Correlation between perfusion variables and histologic findings were tested using Spearman rank correlation in SAS statistical software version 9.2. Results are presented as average (range) unless otherwise stated.

Scoring system for morphologic findings

In the revised scoring system for morphologic findings in normal and in vitro perfused tissue, some entities were registered as ''nonpresent'' or ''present/abnormal.'' For some, ''present/abnormal'' had to be defined. Abnormal villous maturation was registered as ''increased syncytial knots'' when syncytial knots were present on ≥30% of terminal villi in the maternal three quarters of the parenchyma [13] and as ''delayed villous maturation'' when reticular villous stroma and central capillaries were observed in distal villi. The percentage of involved tissue was registered. Other findings such as avascular villi were also noted. Retroplacental hematomas were arbitrarily defined as present if hematomas indenting the decidual plate covering at least 5% of the maternal surface were observed grossly.

Other entities, including maternal (MIR) and fetal inflammatory response (FIR), chronic villitis of unknown etiology (VUE), and decidual vasculopathy, were graded or staged according to the recommendations of Redline and colleagues [28,30,36]. For some findings, including chronic deciduitis, infarcts, intervillous fibrin deposits, intervillous thrombi (also called intraplacental hematomas), calcifications, and in vitro perfusional changes, recommendations for grading/staging were not found in the literature and arbitrary scores were created.

The grades and stages were defined as shown in Table 1.

RESULTS

The morphologic findings were assembled in main entities: inflammatory lesions, maternal hypoperfusion, fetal trombotic vasculopathy, hematomas, others, and ex vivo perfusional changes.

A summary of the pathologic findings in normal and perfused tissue labeled with results of statistical analyses of interobserver variation followed by range and average of scoring is presented in Table 2.

Of the 34 included placentas, 26 underwent successful perfusions and 8 were leaky. Observed changes secondary to the ex vivo perfusion included dilation of fetal vessels, as well as emptiness of fetal vessels and maternal space found as expected in varying degrees in most cases. Trophoblastic vacuolization was interpreted as a sign of tissue damage (Fig. 1), and bacteria in the fetal vessels were surprisingly frequent. The finding of bacteria in the fetal circulation indicated that the addition of penicillin-streptomycin in the perfusion medium is necessary. Subsequent placentas perfused with antibiotic-containing medium were free of bacteria. Selected morphologic findings (VUE, calcifications, chronic deciduitis, abnormal villous maturation, retroplacental hematoma, necrosis of decidua basalis, avascular villi, trophoblastic vacuolization, and the presence of bacteria) were compared statistically to selected perfusion variables (successful perfusion, fetal flow, and fetal leak). The only significant correlation was between the degree of vacuolization and fetal leak. We found no statistical correlation between fetal leakage and the presence of bacteria. We investigated whether the presence of pathologic findings in the cotyledon correlated with the quality of perfusion but did not find any statistical relations. Comparison of the findings in the normal and the perfused tissue showed no statistical difference for VUE, chronic deciduitis, chronic intervillusitis, calcifications, and abnormal villous maturation.

The overall frequency of histologic findings was much higher in the normal tissue. Several lesions appeared frequently and often showed especially poor interobserver agreement. Other frequent findings in the normal tissue not included in statistical analysis for interobserver variation were embryonal rests in the umbilical cord (10 cases) and multifocal or diffuse squamous epithelial metaplasia of the amnionic epithelium (9 cases). Rare findings were reactive amnionic epithelium (3 cases), meconium-laden macrophages in the free membranes (1 case), marginal hematoma (1 case), true umbilical knot (1 case), hypercoiled umbilical cord (2 cases), subacute chorionitis (2 cases, 1 associated with VUE), and acute intervillositis (1 case).

Table 1. Scoring system for morphologic findings in normal and perfused placental tissue

Morphologic finding	Stage 0	Stage 1	Stage 2	Stage 3
Maternal inflammatory response	-	Acute chorionitis/ subchorionitis	Acute chorioamnionitis	Necrotizing chorioamnionitis
Fetal inflammatory response	-	Chorion vasculitis/ umbilical phlebitis	Umbilical vasculitis	Necrotizing funisitis/ concentric umbilical perivasculitis
	Grade 0	**Grade 1**	**Grade 2**	**Grade 3**
Chronic deciduitis	<50 lymphocytes/HPF	≥50 lymphocytes/HPF	Multifocal/few confluent foci	Diffuse/several confluent foci
Chronic villitis	<5 involved villi in few foci	<10 villi/focus in 1 slide (low grade, focal)	<10 villi/focus in >1 slide (low grade, multifocal)	≥10 villi/focus (high grade)
Intervillous fibrin deposits	Increased in <40% of the tissue in all slides	In few foci, increased in ≥40% of the tissue, or confluent foci	Increased in several foci or diffuse	Grossly detected white spotty areas
Intervillous thrombi/ intraplacental hematomas	-	Few <2 cm	Some small or few ≥2 cm	≥5, some >2 cm
Infarcts	-	Villous agglutination/ microinfarcts 3–20 villi	Few <3, <1 cm	several ≥3, ≥1 cm
Calcifications	-	Present in 1–3 of 10 LPFs	Present in 4–8 of 10 LPFs	Present in 9–10 of 10 LPFs
Dilatation of fetal vessels[a]	<50% dilated	>50% dilated	-	-
Emptiness of blood space[a]	Fetal vessels and maternal space empty	>2/3 empty	1/3–2/3 empty	<1/3 empty
Trophoblastic vacuolization[a]	-	Present in <1/3 of villi	Present in 1/3–2/3 of villi	Present in >2/3 of villi

HPF indicates high-power field; LPF, low-power field.
[a]Perfused tissue only.

Redefinition of minimum criteria of frequent lesions with poor reproducibility, abnormal findings, and interobserver agreement results

Despite our initial attempts of precisely defining each histologic lesion, the interobserver variation was poor in several lesions, most of them frequently present. In the joint meetings, it was especially troublesome to agree on precise minimum criteria for these lesions. In an attempt to improve the interobserver agreement, we therefore decided to redefine the criteria for frequent lesions (present in >10% of the cases) initially showing poor or fair agreement in the nonperfused tissue. The frequency of these lesions suggested that they were overdiagnosed, due to a too sensitive initial diagnostic threshold. For each lesion, the distribution of grades or stages and/or findings on re-evaluation was used to define the threshold, above which <10% of the cases were positive. The gross or histologic criterion for this threshold was compared to recommendations in the literature, and a final minimum criterion for each entity was defined. One year after the initial evaluation, the normal tissue in all 34 cases was re-evaluated according to the final minimum criteria. As previously, the gold standard for the presence of a lesion was a consensus diagnosis reached by joint discussion. The exact definitions of the final minimum criteria, the final number of cases with abnormal findings (consensus), and the final results of interobserver agreement are as follows:

Maternal inflammatory response

Diffuse band of neutrophils in the chorionic trophoblast layer and/or subchorion. 0 cases, good agreement.

Chronic deciduitis

Presence of plasma cells and/or lymphocytic infiltration of ≥50 lymphocytes/per high-power field in multiple foci (>2) or diffusely in at least 1 slide. 3 cases, good agreement.

Table 2. Morphologic findings in normal and perfused placental tissue (total $n = 34$, statistical comparison performed on $n = 28$ [see data analysis])

	Normal		Perfused	
	n	**Range (average)**	***n***	**Range (average)**
Inflammatory lesions				
Acute				
Maternal inflammatory response	16[a]	0–1 (0.4)	1[a]	0–1 (0.0)
Fetal inflammatory response	6[b]	0–1 (0.2)	3[c,d]	0–1 (0.1)
Chronic/subacute				
Chronic deciduitis	16[a]	0–2 (0.5)	14[c]	0–2 (0.4)
Chronic villitis	9[b]	0–3 (0.4)	7[c]	0–3 (0.4)
Chronic intervillusitis	0[b,d]		2[b,d]	
Eosinophilic/T-cell vasculitis	3[b]		0[b]	
Maternal hypoperfusion				
Decidual vasculopathy				
Free membranes	2[c,d]	0–1 (0.1)		
Basal plate	0[b,d]		0[b]	
Infarction	3[b]	0–2 (0.2)	0[b,d]	
Abnormal villous maturation	18[a]		9[a]	
Increased intervillous fibrin deposits	10[a]	0–3 (0.4)	3[a]	0–1 (0.1)
Fetal thrombotic vasculopathy	3[b,d]		4[a]	
Hematomas[e]				
Retroplacental hematoma	6		0	
Intervillous thrombi	16	0–2 (0.6)	3	0–1 (0.1)
Others				
Calcifications	28[a]	0–3 (1.1)	26[a]	0–3 (0.9)
Decidual necrosis				
Free membranes	20[a]			
Basal plate	13[c]		7[c]	
In vitro perfusional changes				
Nondilated fetal vessels			5[a]	0–1 (0.1)
Blood in fetal vessels and maternal space			29[a]	0–3 (1.0)
Trophoblastic vacuolization			23[c]	0–3 (0.9)
Bacteria in fetal vessels			15[b]	

[a]Poor interobserver agreement.
[b]Good interobserver agreement.
[c]Fair interobserver agreement.
[d]Invalid kappa; see data analysis.
[e]Gross examination by 1 observer.

Delayed villous maturation

Delayed maturation in ≥30% of villi in ≥30% of the tissue in at least 1 slide. 3 cases, fair agreement.

Increased syncytial knots

Syncytial knots present in ≥30% of villi in ≥30% of the tissue in at least 1 slide. 6 cases, good agreement.

Increased intervillous fibrin deposits

Grossly visible spotted white areas or histologic intervillous/perivillous fibrin deposits multifocally or diffusely occupying ≥40% of the tissue area in at least 1 slide and not qualifying for massive perivillous fibrin deposits. 2 cases, good agreement.

Increased calcifications

Course calcifications visible in low power and present in every low-power field examined in at least 1 slide. 0 cases, good agreement.

Decidual necrosis

Ischemic necrosis presents away from the margin of the placental disc. *Free membranes:* Diffusely present in the decidua capsularis (≥50% of the roll). 1 case, fair

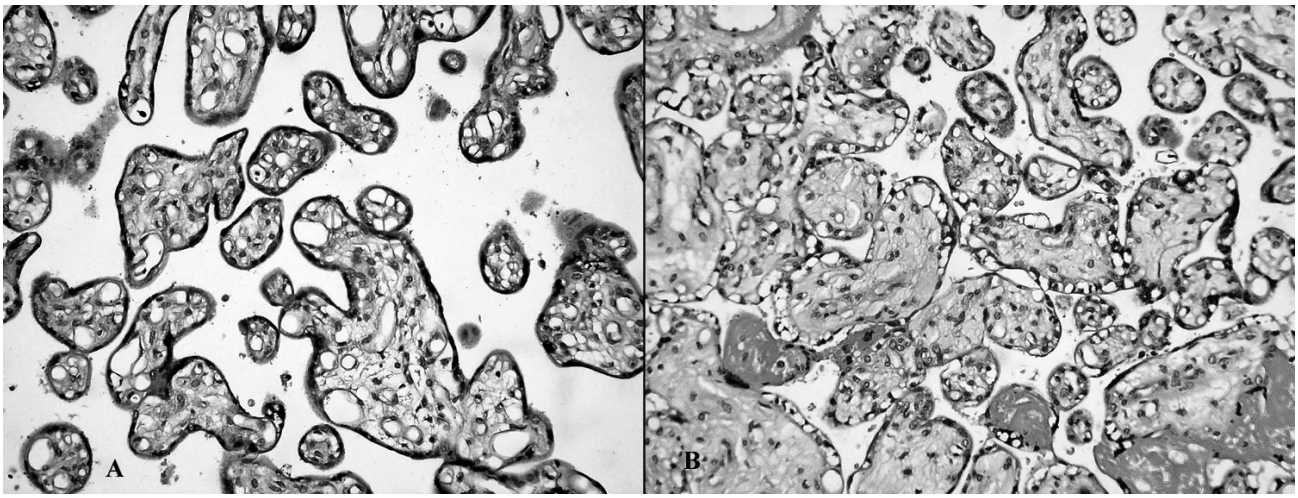

Figure. 1. Perfused tissue with and without histologic signs of tissue damage. **A.** Perfused tissue showing dilatation of fetal vessels and empty capillaries and intervillous space (hematoxylin and eosin [H&E], ×200). **B.** Perfused tissue with trophoblastic vacuolization (tissue damage) (H&E, ×200).

agreement. *Basal plate:* Multiple foci (≥2) measuring ≥1 × 1 mm present in the decidua basalis in at least 1 slide. 2 cases, fair agreement.

More in-depth analysis of newly defined frequent lesions

Maternal inflammatory response

Acute chorioamnionitis is believed to be an accurate indicator of infection in the amniotic fluid and is found in 1%–4% of term pregnancies. The interobserver variation is good in most studies [26,28,31]. The interpretation of maternal inflammation in the free membranes was a subject of repeated discussions during the evaluation process, because a precise definition of slight inflammation vs normal was difficult to reach. The refined threshold used in this study for MIR, score 1 was based on the description in ''Placental Pathology'' [11] and defined as ''neutrophils, not believed to be reactive to non-infectious processes, in the subchorionic fibrin and/or membranes at the junction between the decidua and chorioamnion.'' Despite this, the interobserver agreement was poor and the high frequency of MIR suggests that our criterion was much too sensitive. In the original paper by Redline, the definition is ''patchy-diffuse accumulations of neutrophils in the subchorionic plate fibrin and/or membranous chorionic *trophoblast* layer'' [28]. A re-evaluation of the cases, with emphasis on neutrophils in the chorionic trophoblast layer required, reduced the number of cases with MIR, score 1, to 6 (18%). In a recent paper published after the re-evaluation, the occurrence of ''a diffuse band of neutrophils'' is underlined as a minimum criterion to avoid overdiagnosis of MIR [38]. The final evaluation according to this more strict definition resulted in no detected cases of MIR and a complete agreement between the observers. The ''pitfalls'' in the overdiagnosed cases were mainly focal ischemic changes with neutrophilic debris, subacute inflammation associated with VUE, and focal neutrophilic accumulations due to bleeding. This study underlines the importance of ''diffuseness'' of the infiltrate as a minimum criterion for maternal inflammation.

Chronic deciduitis

T-lymphocytes are a normal part of the decidua making up about 10% of all decidual cells, while B-lymphocytes and plasma cells are virtually absent [14]. Of the 16 cases, 2 were grade 2 lesions and the rest grade 1. Hence, the definition of grade 2 would be appropriate to achieve <10% positive cases. This correlates well with the findings of Khong and colleagues [27].

Delayed villous maturation

Defining abnormal maturation of villi is difficult, and the poor interobserver agreement is not surprising. Villous maturation can be evaluated in different ways. Rather complex scoring systems have been developed based on villous angiogenesis and stem villous fibrosis [14]. Also, the amount of syncytiovascular membranes in the terminal villi can be counted [39]. During the development of the scoring system, we found these evaluation systems subjective and impractical to work with, and they were therefore not used in this study. Premature development of terminal villi as a result of hypoxia cannot be assessed in a term placenta. An easily assessable indicator of hypoxia, however, is increased syncytial knots in >30% of villi [13]. Delayed villous maturation (maturation defect) is difficult to assess and is easily confused with villous edema. Exact definitions from the literature do not include a suggestion for percentage of involved tissue for diagnosis [40]. In the initial evaluation, increased syncytial knots and delayed villous maturation were described in detail for each case, although they were lumped into 1 category for statistical analysis. Of the 18 cases, 13 showed increased syncytial knots in 10%–30% of the tissue, 2 of these in 30% of the tissue area, which is in keeping with the findings of others [30]. Distal villous

hypoplasia was not observed. Delayed villous maturation was found in 6 cases; in 1 of these cases, 30% of the tissue was affected. Therefore, involvement of 30% of the villous tissue was used for the final definitions of increased syncytial knots and delayed villous maturation.

Increased intervillous fibrin deposits

Limited amounts of intervillous fibrin deposits are present in all term placentas, especially under the chorionic plate and marginally. Visible amounts are stated to be abnormal [14]. Increased intervillous fibrin deposits defined as an inappropriate amount of fibrin for gestational age is one of several findings seen in maternal hypoperfusion [30]. We could, however, not find exact definitions of appropriate amounts of intervillus fibrin in relation to gestational age in the literature. Only 2 of the 10 cases showed > grade 1 (1 grade 2, 1 grade 3), so our definition of grade 2 was a useful lower cut-off. The designation ''increased intervillous fibrin deposits'' should, however, not be used if the amount of fibrin qualifies for a diagnosis of massive perivillous fibrin deposits [41].

Increased calcifications

Calcifications are present in irregular distribution in the placental tissue in increasing amounts during pregnancy. The calcifications are rarely visible grossly in premature placentas but are often seen and felt on cut surface in postmature placentas. The significance of placental calcification is largely unknown [22]. In this study, only course calcifications visible on low power were evaluated. They were present in 28 cases (82%), of which 22 were grade 1, 4 grade 2, and 2 grade 3. We used a simplification of our grade 3 definition as the final criterion for increased calcifications.

Decidual necrosis

Decidual necrosis in the free membranes has been reported as one of several findings suggestive of maternal hypoperfusion [30]. In our study, decidual necrosis was very common, more so in the free membranes (59%) than in the decidual plate (38%). Other findings, however, suggestive of maternal hypoperfusion, such as infarctions, distal villous hypoplasia, and decidual vasculopathy, were only found in <10% of the cases. Decidual necrosis was only diagnosed if present away from the placental margin. On review, laminated necrosis of large areas were usually present in the free membranes, while multiple small areas were seen in the decidual plate. We therefore added diffuseness (≥50% of the membrane roll) in the free membranes and multifocality and minimum size (≥2 foci, ≥1 × 1 mm) in the decidual plate away from the margin as minimum criteria for significant decidual necrosis.

DISCUSSION

Normal placentas are usually not sent for pathologic examination in our institutions, and this study therefore offered an excellent opportunity to examine a series of perfused cotyledons as well as nonperfused placental tissue from uncomplicated term pregnancies. The creation of the revised scoring system was a complicated process, especially concerning the definition of thresholds for pathologic findings. Once developed, we found the scoring system easy to follow.

In the perfused tissue, recognition of histologic artefacts due to perfusion vs pathologic histologic changes was not problematic. Of the observed perfusional changes, only trophoblastic vacuolization showed acceptable interobserver variation and was deemed reproducible. It was significantly associated with fetal leakage. Histologic examination of perfused tissue for trophoblastic vacuolization should be considered as an additional quality marker in ex vivo perfusion studies.

The placenta is a large organ with a great reserve capacity, and placental lesions are often incidental and not necessarily associated with complications or adverse outcome of the pregnancy. The definition of a pathologic vs a normal process is especially problematic in the placenta due to its explosive growth and short lifespan. The more frequent a finding is in normal placental tissue, the more problematic it is to define an upper limit of normality. In cases of adverse outcome, several different placental lesions are often found and the clinical influence of each of these is often unknown. Some of the most frequent findings in term placental tissue lack minimum criteria for abnormality in the literature.

In this study, the larger number of histologic findings in normal vs perfused tissue (Table 2) is probably explained by the larger amount of nonperfused tissue examined grossly.

In our experience, some histologic findings, such as calcifications, decidual necrosis, altered villous maturation, and intervillous thrombi, are so common in the daily routine on pathologic placentas, that the high frequency in this study was not surprising. However, other findings, such as chronic villitis in 9 cases, 3 of these high grade, and the rare entity eosinophilic T-cell chorionic vasculitis in 3 cases, were unexpected. It could be speculated that these findings are more common in uncomplicated pregnancies than previously suspected. However, the amount could be coincidental due to the limited total number of cases in this study. We found good interobserver agreement for these lesions, probably because they are well defined in the literature [35,36]. The good interobserver agreement found in the normal tissue for FIR, VUE, and infarction was in accordance with the findings of others [25,26]. Poor interobserver agreement for altered villous maturation has also been reported [24].

The observation of poor interobserver agreement in several frequent lesions led us to investigate the lower limits of these lesions further. The choice of a 10% cut-off for frequency is arbitrary but in keeping with the threshold chosen by others for definitions of, for example, decreased placental disc weight [42] or abnormal umbilical coiling index [43]. On re-evaluation, the final minimum criteria

proved reproducible on interobserver evaluation. An example of a lesion with poor interobserver agreement in this study is MIR. Initially almost half the cases showed stage 1 MIR. That was highly unexpected, because the incidence of chorioamnionitis in uncomplicated term pregnancies is reported as <5%. Also the poor agreement was surprising, and we suspected overdiagnosis due to imprecise criteria for diagnosis. With moderate and severe inflammation, it is easy to define the stages; however, a reproducible definition of slight inflammation vs normal was only reached after 2 adjustments and re-evaluations of the primary definition. This study emphasizes that chorioamnionitis is a diffuse illness and that detection of the diffuseness of the infiltration is mandatory to avoid overdiagnosis.

In this paper, we have focused on the difficulty of defining lower thresholds for certain placental lesions. The major weakness of this study is the limited number of cases and observers, and the suggested minimum criteria for frequent placental lesions presented here should be tested for reproducibility and clinical relevance in larger studies.

CONCLUSION

In this study, the morphology of perfused and non-perfused placental tissue has been evaluated in detail. Trophoblastic vacuolization was the only reproducible perfusion-induced change observed. It was statistically significantly correlated with leakage from the fetal compartment in the perfusion set up. Based on our findings, we recommend histologic examination of the perfused tissue for trophoblastic vacuolization as an additional evaluation marker during introduction of new or revised placental perfusion protocols.

Additionally, the histology and interobserver variation of normal term placental tissue has been evaluated in detail. Agreement among colleagues on histopathologic evaluation of placental tissue is not easily obtained, even in the hands of trained pathologists, especially concerning the threshold for reporting specific findings as abnormal. This study discusses minimum criteria for some frequent placental lesions and underlines the need for exact definitions.

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