



# Implications of placental pathology for disease mechanisms; methods, issues and future approaches



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## ABSTRACT

Pathological examination of the placenta is a well-established investigation following delivery in order to investigate the underlying mechanisms of a range of pregnancy related complications. Several recommendations and guidelines are available regarding the indications for such placental testing. The immediate clinical rationale for this process is to identify underlying disease processes which may have an impact on the management of either the infant or the mother in future pregnancies. Additional benefits include improved understanding of the pathophysiological processes of disease and potential medico-legal implications in cases with adverse outcome, including regarding possible timing of lesions. However, interpretation of findings in specific cases remains difficult for several methodological reasons. Future progress requires the use of high quality, well phenotyped tissue collections, with blinded assessment using consensus criteria. In addition, it is likely that novel discovery-based approaches will significantly change the concept of how placental disease is investigated, making tissue sampling even more important across a wide range of pregnancy-related diseases. This will be associated with more stringent conditions for placental evaluation and sampling, including strict definitions of sample site and interval post-delivery, the effects of which will vary depending on the precise assays and methodologies used.

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## 1. Introduction

Pathological examination of the placenta is a well-established investigation following delivery in order to investigate the underlying mechanisms of a range of pregnancy related complications. Several recommendations and guidelines are available regarding the indications for such placental testing [1,2]. The immediate clinical rationale for this process is to identify underlying disease processes which may have an impact on the management of either the infant or the mother in future pregnancies. However, additional benefits include improved understanding of the pathophysiological processes of disease and potential medico-legal implications in cases with adverse outcome, including regarding possible timing of lesions [3]. Due to the specialist nature of diseases affecting the placenta, such specimens should be examined by dedicated paediatric and perinatal pathologists rather than general pathologists; in one study, 40% of placental reports generated by non-specialist pathologists contained errors, predominantly errors of exclusion but also including false positive diagnoses [4].

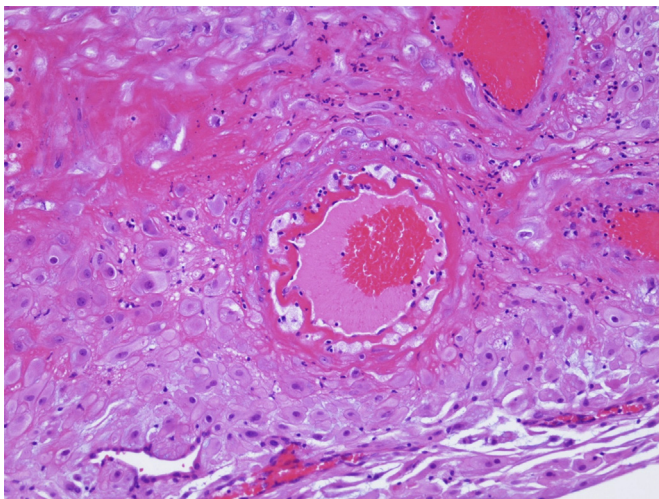
The standard approach to placental examination for clinical purposes includes weighing and measuring of the placenta, (and associated cord and membranes), followed by detailed inspection, and systematic sectioning of the placental parenchyma to identify macroscopic lesions. Tissue blocks are then routinely obtained from the cord, membranes and representative areas of the placenta proper for subsequent formalin fixation, processing, paraffin embedding and cutting for histological examination and reporting. It should therefore be noted that while a wide range of specialist techniques are available, these are not routinely performed. Furthermore, the sampling protocol described above is predominantly for clinical purposes. The tissue requirements for many research studies will therefore likely not be served by routine placental handling protocols used in the clinic. For example, rapidly obtained, snap frozen tissue is not routinely obtained in most clinical services. Specific and targeted placental research projects often require dedicated additional protocols in order to obtain tissue of the appropriate type and quality. Details of the precise sampling protocols for different types of research study are discussed in detail in a recent position paper/guideline in this journal [5].

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## 2. Contributions of placental pathology to disease pathophysiology

The histological evaluation of placentas from different patient groups has illuminated our understanding of the underlying disease processes which may result in a range of clinical phenotypes. Two examples to illustrate this area are preterm birth (PTB) and intrauterine growth restriction (IUGR). Studies of PTB have determined that a major cause is ascending genital tract infection, which has a strong relationship to gestational age; chorioamnionitis affects the great majority (>80%) of mid trimester spontaneous losses and severe early preterm deliveries, whereas this is less common towards term, where it affects around 10% of deliveries [6]. Other causes of PTB include changes of maternovascular malperfusion (MVM; see below), but other cases may demonstrate no significant placental pathology, and are likely a consequence of maternal factors such as cervical incompetence or idiopathic onset of preterm labour [7,8]. The finding that several categories of disease and mechanisms may result in an apparently common clinical phenotype is important, since it allows targeted strategies to be derived specific to each mechanistic group.

Similarly, studies examining placental findings in IUGR across all gestations have reported that around one half of cases demonstrate features of typical maternovascular malperfusion (MVM) secondary to impaired trophoblast invasion with defective conversion of uterine artery branches into low resistance uteroplacental vessels [9,10]; Fig. 1. It is further recognised that there is significant overlap between features of early onset IUGR and early onset pre-eclampsia (PET), sharing common placental changes. More recently, there has been wider appreciation that early versus late-onset IUGR and PET show differing patterns of pathology, with late onset cases often associated with minimal placental histological abnormalities suggesting that these entities may have different underlying mechanisms, with more late onset cases associated with impaired maternal adaptation [11]. Finally, as with PTB, smaller subgroups may demonstrate other, specific, pathologies, such as chronic histiocytic intervillitis or massive perivillous fibrin deposition, whilst others may be associated with no significant morphological abnormalities, their mechanisms remaining uncertain, but for example, being due to impaired transport functions.



**Fig. 1.** Photomicrograph of a maternal decidual vessel demonstrating pathological changes of atherosclerosis, with fibrinoid change and foamy macrophage infiltration of the subintimal region. (Haematoxylin and Eosin, original magnification  $\times 100$ ) These changes indicate significant maternal vasculopathy, which may be associated with a range of underlying conditions and complications such as pre-eclampsia.

## 3. Issues with placental pathology approaches

Despite the undoubted value of placental histological evaluation for recognition of patterns of underlying pathophysiology in groups of patients, there are several difficulties with the traditional approach on which much of the existing data is based. Firstly, placental histological evaluation is in many ways more difficult than other areas of diagnostic pathology, such as oncology for instance, since in the majority of cases, there are few morphological findings that are unequivocally diagnostic of a particular condition or phenotype, and no specific immunostain or routine molecular investigation can provide a definite 'gold standard' diagnosis. For most conditions, such as IUGR, there are few pathognomonic lesions which are never encountered in clinically uncomplicated pregnancies, (with the probable exception of acute atherosclerosis), and most of the findings in IUGR simply occur more frequently, and in different combinations, than in controls [12]. This requires a subjective assessment of the significance of such features in a given case meaning that when evaluating data from retrospective studies it is often impossible to separate the objective findings present from the subjective interpretation of such findings, which may obviously vary according to the reporting pathologist.

Furthermore, in general, for individual cases there is poor correlation between the extent of histological changes and clinical severity of disease. This is likely in part due to sampling issues, but also because of the varied underlying mechanisms and maternofetal interactions which may result in a clinical phenotype. For example, in term PET, even clinically severe disease may be associated with only mild morphological changes of the placenta [13].

### 3.1. Poor clinical phenotypes

A further significant difficulty in interpretation of placental findings is related to the loose clinical phenotypes used for both cases and controls in many historical studies. For example, the majority of the literature regarding IUGR is based on the 'case' group being identified as birthweight  $<10$ th centile, this representing SGA rather than pathological IUGR. Around half of all cases of SGA are likely normal small rather than pathologically growth restricted and hence inclusion of all as 'SGA cases' will by definition include a mixture of normal and pathological pregnancies [14]. In addition, 'controls' are often identified as cases submitted for histological assessment due to a clinical indication which differs from the 'case' group, rather than truly being matched normal controls. This situation is obviously exacerbated in cases with preterm delivery since normal controls largely do not exist.

It is now increasingly recognised that rather than extremely large studies with loose inclusion criteria, higher quality data to answer specific questions can be derived from studies including smaller numbers of patients but with extremely strict entry criteria to ensure that the category of interest is as well represented as possible without 'dilution' by other disease phenotypes [15].

### 3.2. Interpretation of lesions; blinding and bias

Since clinically submitted cases requiring a formal histopathology report require interpretation of findings in light of the clinical information, the majority of such cases are not reported blinded to the patient history or other findings. These factors can significantly influence the content of the report and hence retrospective series of clinical reports provide relatively poor quality data for targeted scientific studies. It has been demonstrated, for example, that dating of placentas is poor even by experts, and that the gestational age stated on the request form has a major influence on the apparent interpretation of gestational age performed by

pathologists, regardless of the histological content of the section [16]. This lack of blinding when reporting is therefore associated with significant inherent bias in interpretation and for scientific studies it is essential identification of findings is performed without knowledge of other information, and areas of interpretation should be clearly distinguished from observations of fact.

Furthermore, for optimal determination of clinical significance of features it is important that placentas are examined from an unselected population so as to ensure both representation of the spectrum of cases with normal outcome, and also to avoid artificially weighting to sample in favour of pathology. A large study in Cambridge, United Kingdom, examined all placentas from a completely unselected population of >1000 low risk pregnancies delivering at or near term [17,18]. All placentas were examined and sampled by trained technicians according to a standard protocol and the sections were reviewed by two pathologists with no clinical information other than study number. In this way, it is possible to calculate odds ratios for associations of patterns of placental pathology and specific outcomes, in a similar way to case control studies, but importantly, such an approach also allows calculation of other 'test' characteristics such as sensitivity and specificity for specific histological findings.

This approach provides an interesting perspective on certain features. For example, villitis of unknown etiology (VUE) was present in around 3% of normal term controls compared to around 10% of those complicated by pregnancy induced hypertension (PIH), giving an odds ratio of around three, consistent to that derived from previous case-control studies and confirming an association between this histological feature and this complication. However, since normal deliveries uncomplicated by PIH were far more frequent than cases with PIH, in an unselected population, the finding of a placenta with VUE has a >92% chance of being an incidental finding from a delivery with no PIH, indicating that interpretation of the significance of findings such as VUE in individual cases is difficult [18]. Datasets that preferentially receive placentas from complicated pregnancies, corresponding to most clinical units, will therefore by definition tend to overemphasise the perceived importance of features compared to datasets examining placentas from consecutive unselected deliveries.

#### 4. Future role of placental pathology assessment

Despite all of the caveats noted above regarding interpretation, there remain important roles for placental histological evaluation for research purposes. First, these morphology-based assessments can be used to broadly categorise the underlying mechanisms associated with clinical phenotypes, such as features of uteroplacental malperfusion versus chronic histiocytic intervillitis in IUGR for example. Such classifications have significant impact on understanding the impact of interventions and may be used to better stratify future patient management [19]. Secondly, this approach will allow increasingly specific determination of phenotypic-pathological correlation. For example, the recognition that placental histological findings in pre-eclampsia vary according to the gestational age at presentation has significantly contributed to the current concept of early onset versus late onset disease representing overlapping, but likely fundamentally different, pathophysiological mechanisms related to placental implantation and maternal adaptation respectively [20].

Nevertheless, to ensure the continued usefulness of morphological, and other, placental evaluation to obstetric medicine requires stringent adherence to the following principles. First, the underlying clinical phenotypes of recruited populations must be extremely well-defined and homogenous; for example, it is unacceptable to report on cohorts of placentas from deliveries which are

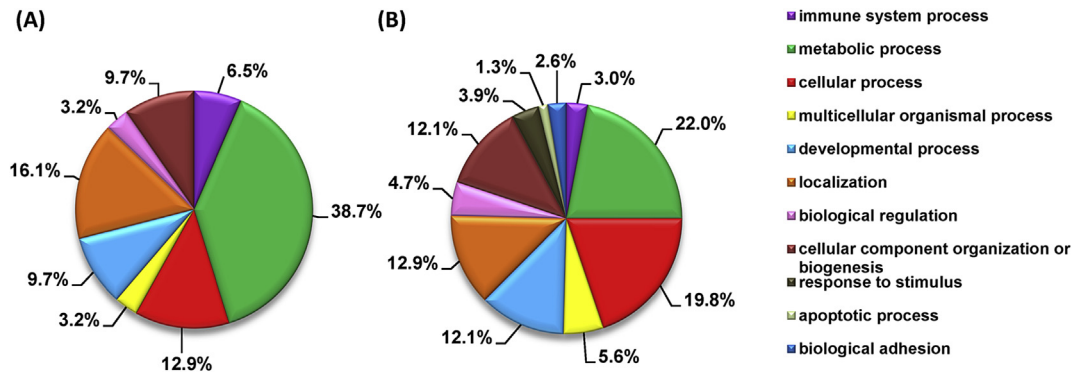
simply defined as 'SGA', which comprise heterogenous groups of both constitutionally small infants and a range of different pathologies. What is required are smaller cohorts but with tightly defined inclusion criteria, (for example early onset IUGR defined as serial confirmation of impaired fetal growth associated with abnormal uterine and umbilical Doppler flow profiles at 24–30 weeks of gestation with or without abnormal maternal serum markers such as PAPP-A). Secondly, there must be use of reproducible and accurate classifications for histological findings to minimise subjective variations in interpretation of features. Initiatives such as the recent Amsterdam Placental Consensus Meeting and associated publications will contribute positively to this area [21]. Thirdly, it is essential that for all research studies, in contrast to clinical reporting, pathologists evaluating placental findings are blinded to the clinical information; ideally blinded to all clinical information not just which group a case belongs to, in order to remove unconscious bias. Retrospective reviews of non-blinded clinical series, whilst providing some useful information, have significant potential for bias and should not be equated with dedicated research cohorts. Finally, the optimal solution would be to, in addition, have a range of novel objective tests for 'pathologies' and their significance rather than sole reliance on subjective interpretation by experts.

#### 5. Future novel approaches

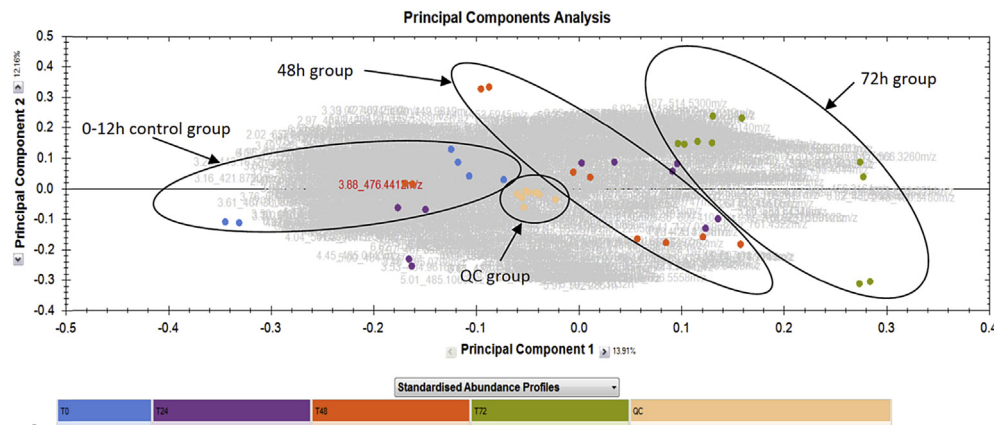
The recognition that interpretation of the significance of various histological findings in individual cases is difficult for the reasons stated above, in conjunction with the lack of direct functional assessment by traditional morphological examination has led to recent interest in development of novel laboratory based approaches to placental evaluation, which will likely have significant future impact on the field.

The main enablers have been recent technological advances in areas of 'discovery based science', particularly genomics, transcriptomics, proteomics and metabolomics [22]. These techniques allow generation of large amounts of data from which novel insights may be obtained without the need for individual hypothesis testing. Since these areas remain in their infancy, particularly with regard to application to the placenta, there remain several practical difficulties in their use, not least in the area of bioinformatics for evaluation of the significance of findings. Nevertheless, these issues are tractable and such approaches are already contributing to our understanding of placental disease. For example, studies examining placental RNA expression profiling (transcriptomics) have reported characteristic expression patterns associated with IUGR with abnormal umbilical artery Doppler waveforms, [23,24]. Similarly, studies examining umbilical cord blood samples have reported changes in metabolic profiles (metabolomics) which allow separation of pathological IUGR from other infant groups with a high degree of accuracy [25,26]. Such findings will not only provide the potential for improved diagnostic testing but also significantly contribute to the understanding of the pathogenesis and mechanisms of such diseases.

However, these novel techniques are associated with additional technical issues which need to be addressed, especially around placental sampling and interpretation. These include determination of the exact anatomical site of samples obtained, their timing in relation to delivery, and the effects of antenatal and intrapartum factors such as mode of delivery. The placental transcriptome has now been described, with significant intraplacental variation between different areas of the parenchyma [27,28]. There are clear alterations in profile between maternal, parenchymal and chorionic plate areas [29], and proteomic studies suggest differences between superficial and deeper, and peripheral and central, areas of



**Fig. 2.** Illustrative example demonstrating the different proportion of proteins involved in various biological process that are detected from the same placental parenchymal sample treated identically but using only different protein extraction protocols. (A&B) [30].



**Fig. 3.** Principle component analysis to assess the distribution of free peptide ions in sample groups according to post-delivery interval showing areas of maximum variation within the data. There is a clear demarcation between control (0–12 h) and 72 h, similarity amongst 48 h samples but with marked variation due to other factors in all groups. QC samples clustered closely, indicating excellent technical quality assurance [31].

parenchyma [30]. Such new methodologies require evaluation for comparative studies since it is recognised that technical aspects, such as variation in protein extraction protocol used, can affect results [30]; Fig. 2. Furthermore, there is to date little data regarding the effects of storage interval (time from delivery to sampling) on the transcriptome, metabolome or proteome. It is well recognised that for optimal RNA extraction, placental tissue should be snap frozen or placed in RNA protection medium as soon as possible, and ideally within 30 min of delivery [5], but the effect of longer intervals on other aspects such as proteomic profile remain to be established, although initial data suggest potential significant effects Fig. 3; [31]. Finally, once the use of such techniques is optimised for application to archival FFPE samples, the vast archives of existing clinical samples may be available allowing novel insights previously not possible from such specimens [32].

## 6. Summary

Traditional approaches of morphological placental examination have significantly contributed to our understanding of many pregnancy related diseases, and this technique will continue to contribute. However, for further future progress there is a need for high quality, well phenotyped tissue collections, with blinded assessment using consensus criteria. It is likely that novel discovery-based approaches will significantly change the concept of how placental disease is investigated, making tissue sampling even more important across a wide range of pregnancy-related

diseases. This will be associated with more stringent conditions for placental evaluation and sampling, including strict definitions of sample site and interval post-delivery, the effects of which will vary depending on the precise assays and methodologies used. Based on these trends, the placenta is likely to provide increasing insights into obstetric-related disease pathophysiology for many years to come.

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