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Current opinion

Optimising sample collection for placental research

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ABSTRACT

Biobanks provide an important repository of samples for research purposes. However, for those samples to reflect the *in vivo* state, and for experimental reliability and reproducibility, careful attention to collection, processing and storage is essential. This is particularly true for the placenta, which is potentially subjected to stressful conditions during delivery, and sample collection may be delayed owing to routine postpartum inspection by clinical staff. In addition, standardisation of the collection procedure enables samples to be shared among research groups, allowing larger datasets to be established. Here, we provide an evidence-based and experts' review of the factors surrounding collection that may influence data obtained from the human placenta. We outline particular requirements for specific techniques, and propose a protocol for optimal sample collection. We recognise that the relevance of these factors, and of the sample types collected to a particular study will depend on the research questions being addressed. We therefore anticipate that researchers will select from the protocol to meet their needs and resources available. Wherever possible, we encourage researchers to extend their collection to include additional samples that can be shared on an international collaborative basis, with appropriate informed consent, to raise the quality, as well as quantity, of placental research.

1. Introduction

Placenta-related biological samples are used in biomedical research to investigate normal placental development, biology, pathophysiology, and the involvement of the organ in developmental programming of adult disease. In order to compare datasets and findings across often heterogeneous studies and cohorts, and for merging of samples or datasets to obtain sufficient statistical power, there is a need for summarising current knowledge regarding methodological factors that affect the quality of samples, and thereby the results. At present, there are no agreed standards for placental sampling or storage of samples, nor for what minimal accompanying clinical information is required for interpretation of the results. It is hoped that an understanding of these factors will improve the quality of individual studies, and also facilitate collaboration across high-quality cohorts and datasets.

No single protocol will satisfy the demands of all researchers, since different research questions may require different samples. Also, limitations in resources and access to suitable facilities may impose restrictions on the range of samples that can be collected across studies. However, our aim is to provide information so that those initiating collections can make informed decisions that optimise their samples for placenta-related research projects, and which may also benefit research areas other than reproductive and offspring health. This information is especially pertinent to The Global Pregnancy CoLaboration (CoLab), which was established in 2011 to facilitate cooperation among researchers who have data and biological sample collections relevant to pregnancy associated diseases (http://pre-empt.cfri.ca/Members/ListofMembers/CoLaboratory.aspx).

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Here, we provide a summary of factors that may contribute to methodological flaws in placenta-related research, owing to variations in sampling and/or processing of tissues. Our conclusions are evidence-based wherever possible, and we highlight areas where further research is required. We first review generic factors that influence data obtained from placental samples, before considering issues that apply to the collection of samples for specific analyses. We then discuss general issues regarding the collection of human samples, and finally suggest a collection protocol that can be adapted to meet the needs and resources available for individual studies.

2. Factors that can influence subsequent analyses

2.1. Maternal characteristics

Various maternal factors can affect placental physiology and pathology, although many have not been studied extensively. A recent publication from the Global Pregnancy CoLaboratory lists the minimal and optimal clinical datasets that should be assembled for studies in pre-eclampsia (Myatt L, personal communication). It is recommended that at least those clinical characteristics in the minimal dataset be collected into a database accompanying the placental tissue collection. Below are examples of non-clinical factors for which relevant data exist.

Ethnicity. Ethnicity may have widespread effects on the placenta through genetic variations, diet and other mechanisms. For example, in a study of almost 700 nulliparous pregnancies, it was reported that both birthweight and placental weight were lower in Asian women compared to European and Afro-Caribbean ethnic groups, although the fetal/placental ratio was not significantly different [1].

Lifestyle. Many studies have investigated the effects of cigarette smoking during pregnancy on placental structure and function [2]. A stereological study found no change in the volume of the placenta or the villous tree, but a significant reduction in the volume and relative surface area of the fetal capillaries [3]. These changes were associated with an increase in the thickness of the villous membrane, and a higher concentration of cadmium within the placenta.

Maternal consumption of alcohol has been linked to a reduction in placental size, reduced blood flow and impaired nutrient transport [4], which may explain the effect on birthweight.

Body mass index. High maternal body mass index is negatively correlated with the fetal:placental weight ratio [1]. This may reflect changes in placental metabolic pathways and amino acid transporter expression [5], possibly induced by the concentration of circulating adiponectin [6]. Maternal obesity is also associated with increased oxidative stress in the placenta [7], and with a global reduction in DNA methylation [8].

Age. A large registry-based study in Norway demonstrated that placental weight increases with maternal age after adjustment for other factors, such as the mode of delivery [9]. Paternal age has a similar and independent effect [10].

Parity. Placental weight is higher in multiparous women than nulliparous women from mid-gestation onwards, irrespective of the fetal sex [11].

Medications. During late pregnancy and delivery pregnant women are variably exposed to a variety of agents including glucocorticoids, oxytocics, anaesthetics, magnesium sulphate, antihypertensives, antibiotics and other agents that may affect placental function. Awareness of these exposures is vital to selection of placentas and avoiding confounding interpretation of data, and a suggested check-list was proposed by Nelson and Burton [12].

2.2. Fetal sex

There is increasing evidence that the sex of the feto-placental unit is associated with profound differences in placental physical parameters [13], function [14] and responses to pathological insults [13,15,16].

Placental weight. Normal ranges for placental weight are established in several countries, and recent gestational age-specific centile charts by sex and parity based on almost 90,000 deliveries have been produced in the UK. These reveal that placental weight and fetal:placental weight ratios are higher in placentas of male than female infants [11].

Gene expression. Microarray analysis shows distinct sexually dimorphic differences in gene expression, with immune genes in particular being expressed at higher levels in the female compared to male placenta [17]. There is also a sex-dependent response in placental gene expression to maternal inflammatory status [18]. Thus, expression of 59 genes associated with growth, inflammation and immune pathways was changed in female placentas of women with asthma vs. no asthma, compared to only 6 genes in male placentas [19]. Changes in diet also generate a distinctive signature of sexually dimorphic genes in the murine placenta, with changes in expression being more striking in female than in male placentas [20]. The male placenta has higher TLR4 expression and a greater production of TNFα in response to LPS than the female, which may underlie the propensity to preterm birth with males [21].

2.3. Mode of delivery

Delivery may be vaginal, either spontaneous or induced, or by caesarean section, either elective or as an emergency procedure following a period of labour. Placentas subjected to labour will be exposed to two principal effects different from those delivered by elective caesarean section; mechanical compression caused by the uterine contractions, and an intermittent reduction in maternal blood supply during contractions [22–24]. The former may impact on quantitative data pertaining to the structure of the placenta, while the latter will influence metabolic data, generation of oxidative and other stresses, and activation of signalling pathways and gene transcription. Variations in the frequency and duration of contractions, and in placental "reserve capacity" will contribute to differences in the degree of hypoxia-reperfusion injury experienced. Unpublished data (Yung, HW) indicate levels of oxidative and endoplasmic reticulum stress in placentas from labour-caesarean deliveries equivalent to a vaginal delivery, even when the period of labour is as short as 2 h.

Other factors associated with the mode of delivery that potentially influence the placenta include whether anaesthesia and/or supplemental oxygen are administered. At present these effects have not been systematically tested, and so researchers may wish to collect relevant clinical information for subsequent analyses. For example, epidural anaesthesia has been associated with reduced utero-placental blood flow; in a controlled study [25], the pulsatility index in the uterine arteries increased 30 min after administration of epidural anaesthesia, but only at the time of uterine contractions. This suggests a potential exacerbation of the ischaemia-reperfusion, even in the absence of neonatal acidosis.

Administration of supplemental oxygen has been shown by cordocentesis to increase the pO_2 in the umbilical vein, implying that the oxygen concentration in the placenta is also increased. This has been confirmed using blood oxygen level dependent (BOLD) magnetic resonance imaging, as the signal increases in the placenta within 2–3 min of maternal hyperoxia [26].

Stereological data. Pressures of up to 100 mmHg are generated within the amniotic cavity during labour [23], and it might be

expected that the placental vascular network is compressed, influencing the dimensions. However, the volume fraction of terminal villi occupied by fetal capillaries was 23% and 29% for vaginal and elective caesarean deliveries respectively in one study, a difference that was not statistically significant [27]. This result was confirmed by a larger study [28] investigating diabetic placentas in which mode of delivery had no effect on villous or vascular dimensions, and only impacted on the diffusion distance of the villous stroma.

Although the mode of delivery *per se* may not compromise stereological data, the timing of clamping of the cord has a profound effect [29]. Comparing normal term placentas in which the cord was clamped early with those clamped after cessation of arterial pulsations, there is a reduction in mean placental weight of >150 g (450 g vs. 640 g), associated with major changes in the values of many parameters, notably placental volume (540 cm^3–388 cm^3), villous surface area (13.3 m^2 vs. 9.3 m^2), volume of the fetal vessels (74.9 cm^3 vs. 33.4 cm^3) and vessel surface area (15.8 m^2 vs. 8.7 m^2). A more recent study reported an average volume for placental transfusion of ~110 ml, usually occurring within 2 min of delivery [30]. Clearly, there is a need to record the timing clamping of the cord if specimens are to be used for quantitative analysis of the fetal vasculature. Perfusion fixation can be employed to redilate the fetal vessels to physiological dimensions [27], but this technique is not suitable for routine collections and may not be compatible with sampling for other analyses.

Metabolomics. The interruption of maternal blood flow during contractions has a profound impact on the metabolic profile of placental tissues. In an early study of normal term placentas, ATP levels measured spectrophotometrically were ~40% lower in vaginal compared to caesarean deliveries, with equivalent changes in energy charge [31]. Surprisingly, levels of glycogen and lactate were similar in the two groups, indicating no compensatory increase in glycolysis. A similar reduction in ATP following labour was reported [32], although no change in activity of complex I of the mitochondrial electron transport chain was observed. This study also reported a reduction in fatty acid oxidation, indicating that this was unlikely to be a significant energy source. In contrast, another study [33] found evidence of increased placental anaerobic glycolysis based on data from nuclear magnetic spectroscopy. Concentrations of glucose and lactate were raised following labour at sea level, along with those of amino acids suggesting that protein catabolism is used as an energy source. There was no change in the ATP/ADP ratio, or energy charge, indicating that adaptive mechanisms were sufficient to maintain the energy balance.

Oxidative stress. Ischaemia-reperfusion is a powerful stimulus for oxidative stress through the generation of reactive oxygen species. One of the principal sources is the enzyme xanthine oxidase, which is proteolytically cleaved from the xanthine dehydrogenase form during hypoxia. It is notable that activity of xanthine oxidase is increased in placentas delivered vaginally compared to those from caesarean sections [34]. A number of markers of placental oxidative stress are raised following labour, including lipid peroxidation, heat shock proteins, expression of the antioxidant enzymes catalase and superoxide dismutase, and activation of the NF-κB and p38 MAPK pathways [35–37]. However, labour has no effect on the superoxide dismutase enzymes in normal placentas, but causes raised levels in placentas from pregnancies complicated by pre-eclampsia [38]. In general, the trophoblast and fetal endothelial cells appear to be the most vulnerable to oxidative stress induced during labour [35].

Gene expression. Given the activation of stress response pathways, changes in gene expression might be expected as a consequence of labour. Several microarray studies have reported both up-regulation and down-regulation of large numbers of genes [35,39–41], although whether the changes are considered statistically significant depends on the cut-off of the fold-change taken, and the *p* value used. Ontogeny analysis assigned the transcripts regulated into the following functional sub-sets: response to stress, cell surface receptor-linked signal transduction, regulation of transcription, immune responses, blood vessel development, cell death, coagulation and anti-oxidant mechanisms. Many genes reported as being changed in placentas from cases of pre-eclampsia, change in the same direction following labour [35]. In addition, the magnitude of the change in selected genes of particular relevance, including *VEGF* and *PlGF*, is related to the length of labour (<5 h or >15 h), and is reflected at the protein level. Expression of *PLAC1* is also reduced following labour [42].

There is currently great interest in placental epigenetics. The recent identification of a novel class of histone demethylases as true dioxygenase enzymes [43] raises the possibility that chromatin structure may change in response to the hypoxic stress associated with labour. Differences in DNA methylation in umbilical cord blood leukocytes obtained from babies delivered vaginally vs. delivered by caesarean section have been described [44], but whether there are changes in placental tissues has not been determined.

Syncytiotrophoblast derived sprouts and vesicles. It has been long known that syncytiotrophoblast derived membrane encapsulated material is deported into the maternal circulation throughout pregnancy. Multinucleate syncytial sprouts are aggregates of euchromatic syncytiotrophoblastic nuclei of ~80–200 μm in size [45]. Due to their size, the majority is trapped in the maternal pulmonary capillaries [46], while smaller syncytiotrophoblast microvesicles pass into the peripheral circulation. This material is composed of different vesicle types, including exosomes (30–100 nm), microvesicles (100–1 μm) and apoptotic vesicles (1–5 μm), together with necrotic debris. Levels of circulating placental vesicles increase with advancing pregnancy and labour, returning to zero in most cases by 48 h post-delivery [47,48].

The stresses of labour trigger endoplasmic reticulum stress and apoptosis, with an associated increase in intracellular calcium that may stimulate microvesicle and apoptotic body release [49,50]. Exosomes are also produced after cell activation, and are secreted from intracellular multivesicular bodies that fuse with the plasma membrane as part of the endocytic pathway [51]. The composition of placental shed material in terms of the proportions of sprouts, exosomes, microvesicles and apoptotic bodies present may have an important bearing on their functional characteristics, with sprouts and large trophoblast debris showing immunosuppressive properties [52,53], exosomes being predominantly involved in immunoregulation and intercellular communication, while microvesicles are more proinflammatory.

Based on the considerations above, we recommend that placental tissue collection for research purposes is performed on non-laboured placental tissue wherever possible, but as a minimum, the duration of labour should be recorded.

2.4. Interval to sample collection

Inevitably, the placenta is exposed to a period of ischaemia following separation from the uterine wall. Hence, the length of the interval between separation/delivery and tissue collection is a critical parameter to be considered.

Placental weight. Stored placentas (kept refrigerated but unfixed) loose ~5% of their weight in the first 12–24 h, and 10% over 48 h [54,55]. This may reflect loss of blood through tears. By contrast, formalin fixation is associated with an average 5% weight gain [56].

Metabolomics. As might be expected, ATP levels drop rapidly after delivery. The volume of maternal blood entrapped within the

delivered placenta has been estimated to contain sufficient oxygen to meet metabolic requirements for 7–10 min, but hypoxaemia occurs shortly after [31]. Loss of cellular energy resources will compromise ATP-dependent activities such as ionic pumping, leading to loss of homeostasis. This is most easily observed at the ultrastructural level, and progressive dilation of organelles, including the mitochondria and endoplasmic reticulum, starts 5 min after delivery [57].

As mentioned earlier, ATP levels are higher in placentas after a caesarean than a vaginal delivery, and so the drop is greater and more precipitate in the former, reaching a nadir after 10 min [31]. Although AMP levels rise markedly in caesarean delivered placentas after delivery [31], activation of AMPK is not seen until between 30 and 45 min [58]. If maternal and fetal blood is washed away then ATP levels drop further, confirming that the blood provides a functional, if short term, reservoir of oxygen. Glucose levels gradually fall by about 30% over 60 min, but glycogen content remains constant. There is a steady increase in lactate. These results were confirmed by a magnetic resonance spectroscopy study of vaginally delivered placentas [59], from which it was concluded that the optimal window for collecting placental samples free from *ex-vivo* ischaemic artefacts is within 10 min after delivery. The same study also reported that increased lipid peroxidation indicative of the generation of oxidative stress is first seen around 20 min after delivery [59].

Signalling pathways. Intracellular energy depletion leads to activation of stress pathways in an attempt to restore cellular homeostasis. Protein synthesis is highly energy demanding, and suppression of translation is a classic mechanism by which cells survive short-term hypoxia [60,61]. Evidence that this occurs in the delivered placenta is first seen 30 min after caesarean delivery, as shown by increased phosphorylation of eIF2α [58].

There are also rapid changes in the AKT/mTOR pathway regulating cell metabolism and growth. Phosphorylation of AKT at Ser473 is reduced by over 40% at 20 min, and then maintained at a low level until at least 45 min [58]. This reflects reduced activity in mTOR, possibly secondary to activation of AMPK. Furthermore, the phosphorylation level of two down-stream substrates of mTORC1, 4E-binding protein 1 (4E-BP1) and p70 S6 kinase, are inhibited by over 40% after 30 min. These changes further suppress protein synthesis.

By contrast, there is no change in levels of the heat shock proteins 27, 70 and 90 over a 45 min period.

Gene expression. Given this activation of signalling pathways, it is likely that changes in gene expression are associated with delayed collection of tissue samples. While one might expect them to follow a similar pattern to those seen following vaginal delivery, experiments to support this assumption are needed.

Stereology. There is a loss of blood from the fetal circulation, presumably through defects in the vascular network but possibly also through changes in vessel permeability. By taking repeated biopsies from caesarean delivered placentas over a 20 min period, it was found that the volume fraction of intermediate and terminal villi occupied by fetal capillaries reduces from 36% immediately after delivery to an asymptote of around 28% after 10 min [62]. This was associated with a rise in the harmonic mean villous membrane thickness from 3.9 to 4.7 μm. Collapse of the fetal capillaries leads to a change in the surface features of the villi seen by scanning electron microscopy, with the formation of furrows in the trophoblast layer as it becomes folded around the diminished villous core [63].

Histopathology. Histopathological changes are surprisingly resistant to delays in fixation, and storage of placentas at 4 °C for 48 h induces minimum artefacts. Changes in the vasculature are seen after 72 h, however [64].

In view of these data, we recommend that samples are collected as quickly as possible after delivery, and that if researchers suspect their analyte is influenced by hypoxia, they should restrict their experiments to samples collected within 10 min of caesarean delivery.

3. Trimming of membranes and placental weight

The umbilical cord and the membranes contribute significantly to overall placental mass, and trimming must be considered before weighing the placenta. In a study of 50 placentas, the median difference in weight between untrimmed and trimmed placentas was 16%, irrespective of gestational age or birthweight [65]. A review of 17 studies reported that untrimmed placentas weigh on average 130–190 g more than trimmed placentas, and therefore the placental; birthweight ratios differed by 0.2–2.34 between trimmed and untrimmed placentas [66].

Therefore, if we assume an average placental weight of around 500 g at term, the variation may be up to 20% depending on timing of cord clamping (see previous section), and around 15% depending on whether complete or membrane-trimmed, with smaller effects of maternal characteristics, delivery mode and storage time. Awareness of such factors is clearly important when interpreting studies regarding significance and relationships of placental weight to other factors.

Taken together, trimming of the membranes is recommended in order to reduce this variability, and to provide a more accurate reference weight for the placental disc.

4. Sampling from the placenta and related structures

4.1. Recording placental shape

Before biobanking placental samples, the gross morphology of the placenta should be detailed and recorded. Several studies have attempted to provide objective measurements of placental shape and cord insertion site, rather than using vague descriptors such as ‘central’ or ‘marginal’. The correlation between digital and manual measurements is good [67]. Centrality and eccentricity indices were calculated using digital techniques in a study of ~1000 unselected women with singleton pregnancies delivering at 37–42 weeks. Average cord insertion was most commonly ‘off centre’, and the placental shape slightly elliptical, with no relationship to common adverse clinical outcomes or histological findings [68,69]. Other studies have also reported that the site of umbilical cord insertion is not associated with changes in placental histology [70], although changes in gene expression have been reported [71].

Conversely, variability of placental shape may be associated with lower functional efficiency [72], and modelling studies suggest that irregular placental outlines and cord insertions may reflect sub-optimal branching of the vascular tree and reduced transport efficiency [73,74]. Changes in placental shape and surface area may reflect the extent of trophoblast invasion and events during the transition from the histiotrophic to haemotrophic phase of nutrition [75]. They may also be influenced by maternal nutrition and periods of fasting [76,77]. Such changes are of interest as they may be associated with developmental programming of increased risk of hypertension and colorectal cancer, and reduced lifespan, in men [78–80].

In practice, a digital photograph of the chorionic and basal plates (including a scale bar) is the easiest method for capturing placental shape, site of cord insertion and chorionic vascular pattern that can be analysed subsequently.

4.2. Bacteriology

In general, microbiological placental sampling has limited value in clinical or research practice, since the placenta may become colonised by vaginal organisms during delivery or following rupture of the membranes. Even when sampling techniques are modified to minimise such contamination, there is generally a poor correlation between microbiological and histological or neonatal findings. Whilst significantly more organisms are detected from cases with chorioamnionitis compared to controls [81], there is a stronger association between detection of organisms and/or inflammatory markers in amniotic fluid obtained at amniocentesis compared to placental cultures [82]. Furthermore, research studies have suggested that many bacteria recovered in such cases are either anaerobes with strict culture requirements or other organisms that may be difficult to culture, such as mycoplasma species [83].

The method of sampling when placental microbiological studies are indicated is important. In one study, cultures of the subchorionic fibrin layer of the placenta were compared to conventional swabs from the surface of the fetal membranes in 79 cases, with subchorionic cultures resulting in significantly reduced contamination with vaginal flora [84]. Even when appropriate sampling methods have been applied, the relationship between microbiological cultures and histological or clinical features remains uncertain. For example, in a study of 376 placentas, histological chorioamnionitis was present in around 25% of cases, but only a quarter of these were associated with positive bacteriological cultures (the proportion being greater in those with funisitis). Conversely, bacteriological cultures were also positive in almost 20% of those with no inflammation present [85].

Since ascending genital tract infection is a major cause of severe preterm delivery, there is an association between placental cultures and gestational age. Several studies have reported that even when placental samples are taken under clean conditions immediately after delivery, cultures are positive in around 50% of preterm deliveries compared to about 25% of those delivering at term [81,86]. Furthermore, there is an association between prevalence of microbial infection of the amniotic cavity and presence of labour.

In conclusion, appropriate interpretation of microbiological placental findings is difficult and dependent on many confounding factors. Research studies specifically investigating this area require rigid protocols regarding both inclusion criteria for cases and precise, reproducible sampling methodologies.

4.3. Placental disc

Tissue samples from the delivered placenta may be used for a wide variety of investigations, and both the optimal number and the anatomical sites of such samples vary according to the specific issue to be addressed. Therefore, it is important to build the sampling requirements into the experimental design.

Sampling may be either regional or global [87]. If the aim is to compare variables between different regions of the placenta to test a specific hypothesis, then those regions must be defined appropriately. However, if the aim is to generate data that represent the whole organ then global sampling must be employed. For example, data obtained from one sample taken in the centre of a placenta and one in the periphery cannot be simply averaged, as the peripheral region of a disc occupies a greater percentage of the overall volume, and so requires greater representation in sampling. Methods for selecting random sampling sites have been described in detail previously [88].

Deciding on the placental sampling site is complicated by the finding that many lesions may be focal and therefore missed if not recognised macroscopically. Conversely, sampling of an established lesion, such as an infarct, may provide little useful information relevant to the surrounding viable parenchyma. For these reasons, both random errors and/or bias may be introduced by the sampling protocol [88]. Furthermore, there is marked variation in placental morphological and histological findings within a given clinical phenotype group, further compounding issues of sampling and interpretation of findings. For example, in a study of 350 placentas from patients with pregnancy-induced hypertension, maternal vascular changes of atherosis and fibrinoid medial necrosis were found in only 20%, despite similar clinical severities of disease [89].

There are several published guidelines for placental examination and protocols for sampling [90–92], but these are primarily aimed at diagnostic histopathologists providing clinical reports and are predominantly based on current practice and opinion (Grade 5/D evidence level; http://www.cebm.net/?o=1025) with surprisingly little reference to published data. Despite their publication, these protocols are generally poorly followed in routine practice, and therefore caution should be exercised in extrapolating findings from retrospective studies from routine diagnostic laboratories. In one study of >600 singleton survivors with cerebral palsy, placentas were submitted for pathologic examination in only 150 (24%) cases [93], whereas in a study of almost 1000 deliveries in a tertiary maternity hospital, <20% of placentas were examined histologically, although 50% should have been according to published guidelines [94].

Several issues are germane for placental sampling for histology. First, how heterogeneous is the regional distribution of histological lesions? Second, is the clinical significance of these lesions the same when found in different areas? Third, what proportion of clinically significant placental lesions are identifiable macroscopically and of those that are not, what proportion of the parenchyma is affected, i.e. how many 'random' samples are needed to be >95% certain that a significant lesion has not been missed?

Whilst it is recognised that there are variations in morphology in different placental regions, there is remarkably little evidence demonstrating the optimal site and number of parenchymal sections needed for defining pathological processes, particularly those with no macroscopic correlates, such as villitis. Regional variations reflect the lobular architecture of the placenta, which in turn reflects the pattern of maternal blood flow [56]. Lobules may be 1–3 cm in diameter, with larger ones near the centre of the placenta. Maternal arterial blood delivered into the middle of a lobule percolates through the intervillous clefts, and drains into the peripherally situated uterine veins [95]. As a result, an oxygen gradient most likely exists from the centre to the periphery. Although confirmatory pO_2 measurements are not available, differences in antioxidant enzyme expression and activity support the hypothesis [96]. The gradient may explain differences in the maturity of the villi, their vascularity, and hence in the thickness of the villous membrane [97–101]. Equally, at term, syncytial sprouts account for about 5% of overall placental volume, but this value varies according to site; lowest values being in the central-parabasal zone where oxygenated blood enters, and highest values being in peripheral "venous" regions [102].

The importance of variations in maternal blood flow to regional sampling was also demonstrated by a study examining villi from nine different sites from six term placentas after uncomplicated pregnancies, sampled from the centre to the lateral edge and basal to chorionic plates. In this study, relative gene expression at each site and villous histology were recorded. Expression of a range of factors differed markedly according to site. For example, certain factors such as vascular endothelial growth factor (VEGF) and connective tissue growth factor (CTGF), were increased in the subchorionic lateral sites associated with increased villous maturation,

syncytial knots and more frequent fibrin deposits, which correlated with up-regulation of hypoxia-related transcripts [71]. Similarly, vascular ACE activity in multiple sites from 12 term placentas increases from the cord insertion site to the periphery [103]. Regional differential gene expression patterns have also been reported in placentas from IUGR pregnancies; in one study of 22 cases, mRNA expression of IGFBP-1, CRH and leptin was increased in the intermediate placental region [104].

Finally, interpretation of histological or gene expression changes should be carried out with great caution following preceding intrauterine fetal death, since many secondary changes occur following cessation of the fetoplacental circulation, and are poorly related to the time interval [105].

We recommend that at least 4 samples are required per placenta to generate 'representative' data.

4.4. Membranes

Optimal membrane sampling protocols depend on the purpose of sampling, in particular, whether the aim is to maximise detection of ascending inflammation or to examine elements of the maternal/decidual vasculature. A commonly suggested protocol, based on pathologists' consensus opinion, is to examine a membrane roll from rupture site to placental edge. (http://www.rcpath.org/Resources/RCPath/Migrated%20Resources/Documents/G/g108_tpplacenta_sept11.pdf). An easy way of performing this in a standardised fashion has been described [106]. Since ascending infection almost always begins in the region of the cervical os, a more optimal protocol for detection of chorioamnionitis is to maximise rupture site sampling [107].

By contrast, several studies have investigated the examination of membrane sections for changes associated with maternal vasculopathy. One study compared standard membrane roll sampling with a protocol which also included a block of multiple flat membrane leaves stacked and serially sectioned. In 120 cases of pre-eclampsia (80 with standard and 40 with modified protocol), atherosis was detected in 25% more cases using the second protocol. Evaluation of traditional placental blocks was associated with atherosis detection rates of <5% for central placental blocks, 15% for placental edge blocks, 25% for membrane rolls and 50% for multiple membrane stacks [108]. Similarly, in a study of 350 pregnancy-induced hypertension placentas, atherosis was detected in 20%, mainly in membrane rolls rather than placental maternal surface sections [89]. Finally, another study examined the utility of taking membrane rolls from all four quadrants and reported that whilst only 4/53 cases had atherosis, the detection rate increased with the number of quadrant sections examined; a single membrane roll identified only half of atherosis compared to all four quadrants [109]. The fact that these elements of the chorionic laeve have very different function from those in the placental bed has not been evaluated and the relative clinical significance of such changes remains uncertain.

4.5. Umbilical cord

The umbilical cord is an important source of relatively pure fetal cells that can be used for DNA typing, freer from maternal contamination than the placental disc. In addition, cord blood is a source of biobankable fetal stem cells.

Many placenta-related research studies aim at matching umbilical cord blood samples to the collected placental tissue. Cordocentesis is rarely justified for research purposes alone, and therefore cord blood is usually sampled after delivery. If the objective is to measure blood gases that reflect the state of the fetus pre-labour rather than that at delivery, or if oxidative stress may affect the substances measured in the fetal circulation, then elective caesareans would be preferable. Also, whether one can start sampling the blood before the arterial pulsations have stopped depends on the clinical situation and prematurity of the offspring. The time to blood sampling follows the general principle of "the faster, the better", but limited studies have tested the time effect on various processes. Separate samples of umbilical arterial and venous blood are usually preferable, as they can identify a fetal or placental source of a biomarker, and discriminate a fetal from a placental condition (such as in fetal cord blood sampling for evaluating neonatal acid–base balance).

4.6. Placental bed sampling

Examination of the placental bed can yield information on the extent of trophoblast invasion, the number and distribution of maternal immune cells, and the extent of remodelling of the spiral arteries. Placenta bed tissue has traditionally been obtained by punch or knife biopsies, either during caesarean delivery, or transvaginally after ultrasound localisation of the placenta prior to termination or delivery [110]. As defined by Brosens and Khong [111], "criteria to confirm placental bed origin of the biopsy include the presence of interstitial, endovascular or intramural trophoblast and/or an artery with physiological changes or an artery larger than 120 μm. However, the absence of both criteria does not necessarily exclude placental bed origin". Placental bed biopsies that contain myometrial tissue are preferable when studying spiral artery remodelling, as the myometrial parts of the arteries are often inadequately remodelled in pre-eclampsia and IUGR.

However, if one wishes to study the decidual basalis alone the vacuum suction technique provides a superior sampling method [112,113]. The collection is performed at caesarean section before the onset of labour, and the uterine wall is subjected to vacuum suction after gentle delivery of the placenta. Advantages compared to traditional biopsies from the placental bed and maternal surface of placenta include; 1) a larger tissue yield, which makes it possible to undertake both morphological and molecular studies; 2) decidual tissue is collected from the *whole* placental bed in an unbiased way; 3) it is easy and rapid and does not lead to short-term or long-term complications when performed by experienced members of staff [114]. One drawback is that the tissue lacks orientation, in contrast to placental bed biopsies.

4.7. Uterine venous blood sampling

Uterine vein blood samples give insight into the specific contribution of placental secretions into the peripheral maternal blood. Blood can be taken at caesarean section from a superficial vein in the lower lateral angle of the uterine segment before uterine incision. If the placenta is lateralized (as predicted by ultrasonography), the placental side is chosen. The vein is cannulated by a 21-gauge needle, and 20–30 ml can be drawn into standard blood collection vials. Since differences between uterine and peripheral vein blood samples are usually studied, an equivalent blood sample is drawn from a peripheral vein within 10 min before the uterine vein sample, and processed in an identical and prompt manner. Haemostasis of the puncture sites needs to be assured before completion of the surgical procedure.

5. Sampling maternal DNA; blood and saliva

Obtaining a sample of maternal DNA is important for performing familial genetic studies relating to placental development. Nowadays, DNA can be isolated from any biological material, and the recovery and amplification of nucleic acids from different

Table 1
Suitability of placental samples for biobanking depending on the mode of delivery, timing of collection and assay to be performed.

Samples for		Non-laboured			Laboured		
		10 min	<1 h	<12 h	<1 h	<12 h	<48 h
Placenta	Histopathology	√	√	√	√	√	√
	Immunohistochemistry	√	√		√		
	Electron microscopy	√					
	Stereology	√	√				
	DNA	√	√	√	√	√	√
	DNA/histone methylation	√	ND	ND	ND	ND	ND
	RNA	√					
	Phosphoproteins/signal transduction	√					
	Protein	√	√	√			
	Metabolomics	√					
	Mitochondrial respirometry	√					
Membranes	Histopathology	√	√	√	√	√	√
	Immunohistochemistry	√			√		
Umbilical cord	Histopathology	√	√	√	√	√	√
	Fetal DNA	√	√	√	√	√	√
	Cord bloods	√					

ND: no data available.

sources, including archived dried blood spots, frozen serum or plasma, long-term stored whole blood is a growing field in retrospective genetic studies [115,116].

In the past, genomic DNA has typically been obtained from anticoagulated blood samples (heparin is not recommended because it can inhibit Polymerase Chain Reaction (PCR) or Restriction Fragment Length Polymorphism (RFLP) analysis). Whole blood samples may be stored for a maximum of three days at 4 °C, or several years frozen (−20 °C or −80 °C). Blood samples can also be collected using FTA (FAST Technology Analysis) paper, and are extremely stable at room temperature.

Saliva is a good alternative source of human DNA [117], and is made attractive by the availability of inexpensive commercially available kits [118]. There is no detectable influence of storage time at 37 °C on the amount of DNA extracted, and even samples kept for 30 days at 37 °C yield approximately as much DNA as fresh samples [119]. Thus, saliva samples are well suited to field conditions.

6. Specific requirements and practical issues

6.1. Obtaining fetal DNA, RNA and protein from placenta tissue and cord blood

The placenta contains high levels of RNases. Therefore, speed is of the essence in preserving the transcriptome, and specimens for RNA should be prioritised. Molecular analyses are strengthened by the comparison of RNA and protein data, and so it is important that the respective samples are taken from adjacent areas. Thus, it is often better to take an initially larger sample (we recommend approximately the size of a grape, 1–2 cm diameter) and then subdivide this into smaller samples for DNA/RNA (5 mg each) and protein (50 mg). It also valuable to fix part of the sample for histological analysis, so that the cellular composition of the tissue can be quantified if needed.

Most protocols recommend rinsing of the sample in buffer at 4 °C before further processing. Rinsing has many benefits, in that it removes most of the maternal blood that is otherwise is a major confounder when equalising protein loading during gel electrophoresis. In addition, it removes the majority of, but not all, maternal leucocytes, which may be considered contaminants in RNA or protein analyses of placental tissue. Nonetheless, a significant number of nucleated maternal cells will remain, either entrapped within fibrin plagues or having migrated into the villous core at sites of syncytiotrophoblast damage [56]. However, rinsing has potentially adverse physical effects, for even gentle agitation can cause the detachment of villous sprouts and trophoblastic debris. Villous sprouts are transcriptionally active, and contain high levels of transcripts encoding sFLT [120]. Consequently, the level of mRNA encoding sFLT is lower in rinsed compared to fresh placental samples from pre-eclamptic patients [121].

Once rinsed and dabbed dry, the samples for protein analysis should be snap frozen in liquid nitrogen as quickly as possible (see Table 1). For protein analysis, multiple samples from the same site (50 mg each) should be spaced around the wall of a cryo-vial rather than as a single clump in the bottom. This allows individual samples to be retrieved without the remainder undergoing freeze-thawing-refreezing. It is best if the 4 samples are kept in separate cryo-vials so that analysis of placental variability can be performed if required. Otherwise, when performing molecular analyses it is most cost-effective to pool samples from each of the 4 sites and generate a global estimate for the analyte concerned.

For DNA, RNA or micro-RNA analysis, samples (5 mg) can be frozen as for protein, but better results are obtained if placed in 1 ml RNAlater. If RNAlater is used, then the samples must be rinsed beforehand as large quantities of maternal blood can inactivate the reagent. Samples should then be stored at 4 °C for 48 h to allow the RNAlater to penetrate the tissues, followed by freezing at −80 °C.

6.2. Histopathology and immunohistochemistry

For histopathological assessment, at least four areas of the placenta that include both fetal and maternal surfaces should be obtained. These are usually 3–5 mm in depth and around 2 cm in width to allow routine processing in 'tissue-tek' type cassettes. Samples are routinely fixed for 24–48 h in neutral buffered formalin before processing and embedding into paraffin wax blocks, from which formalin-fixed paraffin embedded (FFPE) histological sections can be obtained. Longer fixation times may adversely affect future uses of the tissue, such as for protein extraction and immunostaining. Once in FFPE blocks, samples may be stored indefinitely at room temperature.

An alternative is to use methacarn (60% (v/v) absolute methanol, 30% chloroform and 10% glacial acetic acid) as a fixative [122]. Methacarn is a protein-precipitating and non-crosslinking fixative that preserves tissue morphology yet enables high quality DNA, RNA and protein to be extracted from paraffin-embedded material. Consequently, it allows immunohistochemistry, microarray, RT-PCR or Western blotting to be performed on small samples

microdissected from larger blocks of fixed tissue [122]. The solution has been applied successfully for immunohistochemistry of the mouse uterus and placenta at mid-gestation [123].

6.3. Frozen sections

Frozen sections preserve the greatest antigenicity, although preservation of tissue architecture is suboptimal compared to FFPE sections. Small samples (pea-sized; <1 cm diameter) should be washed in cold PBS, dabbed dry and then placed in OCT or similar cryo-medium. Aluminium foil cups formed by folding around the blunt end of a pencil can be used. These can be snap-frozen in liquid nitrogen and stored at −80 °C.

7. Electron microscopy

The size of the sample should be kept small; for transmission microscopy it should be 1–2 mm^3, and for scanning microscopy it can be up to 2–3 times that size. For scanning microscopy, the samples should be washed briefly in physiological buffer to remove serum proteins that may congeal and hide surface details [63]. For routine microscopy the fixative should be 2% glutaraldehyde and 2% formaldehyde in neutral buffer, with at least 10× the volume of solution to tissue to ensure an adequate supply of aldehydes for cross-linking. Leave the specimens immersed in 4 °C for between 4 and 12 h depending on their size. Samples for TEM should be changed to buffer and stored at 4 °C, before post-fixation in 1% osmium tetroxide and embedding in an epoxy or methacrylate resin. Samples for SEM can be stored in fixative at 4 °C. before critical-point drying.

Samples for immunogold labelling should be fixed in either 2% or 6% formaldehyde alone to preserve antigenicity. They should be diced to <1 mm^3 and fixed at 4 °C for no more than 2 h. The stronger fix will give better resolution of structure but the weaker one may preserve sensitive epitopes better. We routinely embed in Lowicryl HM20 at low temperature using the freeze-substitution technique [124].

7.1. Metabolomics

No special treatment is needed for the samples, except for washing in PBS to remove maternal blood. They should then be frozen in liquid nitrogen and stored at −80 °C. A tissue sample of ~5 g was taken by Serkova et al. [59] for high resolution magnetic resonance spectroscopy (^1H and ^{31}P MRS), whereas smaller sizes were used for gas chromatography-time of flight-mass spectrometry (GC-TOF-MS) [125]. Multiple small samples can be frozen in a single cryo-vial, as for protein analysis above.

7.2. Mitochondrial respirometry

Measuring oxygen consumption in the presence of different substrates provides the ultimate physiologically-relevant assessment of mitochondrial function [126]. Mitochondria are, however, vulnerable to *ex vivo* ischaemia and to artefactual disturbances during isolation. A technique for cryopreserving placental villi has recently been developed that enables samples taken immediately after delivery to be analysed later [127]. Villous samples of ~10 mg should be washed in PBS and then immersed in ~200 μl of cryopreservation medium (0.21 M mannitol, 0.07 M sucrose, 30% DMSO, pH 7.0). After 30 s the tube should be frozen in liquid nitrogen. Upon thawing, the cell membranes are permeabilised with saponin, allowing mitochondrial function to be assessed *in situ*. We have found that freezing samples at the point of delivery using this technique preserves mitochondrial coupling better than transporting the placenta to the laboratory on wet ice [127].

7.3. Stereology

Stereology enables data on three-dimensional objects to be generated from two-dimensional sections. It is essential that if total values representative of the organ are to be estimated the overall volume of the placenta, the reference space, is known [128]. Placental volume may be measured in a variety of ways, but in the context of sampling the placenta for multiple purposes the easiest method is to weigh the trimmed placenta and calculate the volume using a specific gravity of 1.05 [129].

When excising the blocks there should be as little compression of the tissues as possible to avoid squeezing blood from the intervillous space and influencing the volumetric composition of the samples artefactually. Use of disposable brain dissection knives, which are designed for soft, friable tissues, is recommended.

Attention needs to be paid to the embedding medium to provide the resolution required. Paraffin embedded material enables a large block face to be cut, providing a large area of the placenta to be sampled. It is also relatively cheap, easy and the sections can be stained immunohistochemically to highlight cell types of interest. However, they are subject to a high degree of shrinkage that must be accounted for, and the resolution may be limited depending on section thickness. Alternatively, resin embedded material can be sectioned at 1 μm, providing high resolution, and is associated with minimal shrinkage [87]. The disadvantages are that the block face is very small, and processing and cutting the samples are more difficult and costly. A combination of the two methods has been proposed, with paraffin sections being used to estimate the main structural parameters, and resin sections for the detailed analysis of intermediate and terminal villi [130]. In practical terms, the same resin blocks may be used as for electron microscopy.

In addition, the orientation of the sections needs to be decided. In essence, one can take 'vertical' sections in a known plane, or randomly orientated sections (isotropic uniform random, IUR). Both are valid approaches, but the probes applied to these sections are different [131]. For the placenta, the chorionic plate provides an easily identified horizontal plane, and vertical sections can be cut perpendicular to the plate. Again, in practical terms it is likely that any blocks taken from chorionic to basal plate for histopathological diagnosis will meet the requirement for vertical sections. To generate IUR sections, the orientator technique can be used [131,132] prior to embedding in wax.

Several reviews of placental stereology have been published, and should be consulted for details of what parameters can be estimated [2,87,88,133].

7.4. Isolation of placental cell types

Protocols have been developed for the isolation by proteolytic digestion of placental cytotrophoblasts, fibroblasts, endothelial cells and macrophages from first trimester and term placentas with a high degree of purity [134–137]. Extravillous trophoblast cells, decidual stromal cells and maternal uterine natural killer cells can also be isolated from first trimester termination material [138]. The isolated cells are usually used fresh, but can be frozen in 10% DMSO at −80 °C and stored under liquid nitrogen.

7.5. Syncytiotrophoblast derived sprouts and microvesicles

To prepare syncytial sprouts and vesicles, placentas from non-labouring caesarean sections should be used where possible. The effects of oxidative stress following placental delivery can be

reduced by the addition of antioxidants into transportation buffers [139]. Placentas should be processed quickly to allow blood to be washed away, thus avoiding excessive contamination of the preparations with non-placental vesicles. Finally, care should be taken to avoid using buffers containing high levels of particles that will be indistinguishable from microvesicles and exosomes, and will lead to overestimated concentration values and interference during size measurements. All media should be prepared by filtration through a 0.1 μm filter, and serum for media supplementation should be ultracentrifuged. Also equipment such as culture plastic, tubes and tubing should be free of particulate material.

7.5.1. Methods to prepare syncytiotrophoblast sprouts

Syncytiotrophoblast sprouts have been harvested *in vitro* from cultured placental explants [120,140]. Chorionic tissue is washed in ice-cold isotonic buffer before being dissected into explants ranging in size from ~10 to 40 mg and cultured for varying periods of time. Cultures should be limited to a maximum of 7 h as by 24 h in culture the syncytiotrophoblast layer has begun to degenerate [141]. Syncytiotrophoblast sprouts can also be collected by placental lobe perfusion, covered in more detail in dual-perfusion below. Syncytial sprouts will be washed from villi into the maternal perfusate. For both explant supernatants and maternal perfusates, syncytial sprouts can be isolated by pelleting using low speed (~800× *g*) centrifugation [47,120,140,142–144].

7.5.2. Methods to prepare syncytiotrophoblast vesicles

There are four main methods for preparing extracellular vesicles from the placenta:

Mechanical separation. Minced chorionic villi are stirred in physiological buffer and the vesicles released are harvested [145]. While this method gives high yields of syncytiotrophoblast vesicles, the need to cut through the tissues may also lead to vesicles from placental endothelial and stromal cells contaminating the preparations.

Explant cultures. Explants, prepared as above, release placental vesicles into the culture medium [142,146]. While this preparation appears to be more biologically relevant than the mechanical method, demise of the syncytiotrophoblast and the presence of non-trophoblast cell types in the explants, which may shed contaminating vesicles into the culture medium, may compromise the preparation's quality.

Placental perfusion. Placental vesicles may also be produced by dual-perfusion described in detail below, and isolated from the maternal perfusate [47,142–144,146]. This method gives high yields of syncytiotrophoblast vesicles, but the unavoidable presence of maternal blood will lead to contamination by predominantly red blood cell vesicles. Purity is also compromised by leakage of perfusate from the fetal to the maternal side. The integrity of the perfused lobe can be monitored by collecting the fetal effluent, with 80% or higher being deemed acceptable [47,142–144].

Primary trophoblast culture. Exosomes and microvesicles are released by cultured cytotrophoblast and syncytiotrophoblast [146,147]. Highly pure mononuclear cytotrophoblasts can be isolated from placental tissue using trypsin/DNAse digestion followed by Percoll gradient separation as described earlier [144]. In culture, these cells undergo rapid fusion to form multinucleated syncytial masses. Therefore highly pure trophoblast derived vesicles can be retrieved, with low levels of contamination from non-trophoblast cells.

For all described methods, once released into the surrounding media, syncytiotrophoblast microvesicles and exosomes can be isolated using sequential centrifugation. A low speed (800–1500× *g*) centrifugation is carried out initially to remove cells and large debris. The supernatant can then be ultracentrifuged to pellet both microvesicles and exosomes [144,148]. If exosomes alone are required, then sequential centrifugations of 800–1500× *g* then 3000× *g*–10,000× *g* should be carried out to remove large debris, cells and microvesicles prior to ultracentrifugation (100,000× *g*) to pellet the remaining exosomes [146]. Exosomes should be further purified in dextrose or Opti-Prep continuous gradient ultracentrifugation, and individual fractions that are enriched for trophoblast-derived exosomes identified using western blotting and a nanoparticle tracking instrument.

It is important to note that vesicles prepared by the different methods outlined above are not identical. They have been shown to have different functional properties [142]. The researcher should choose a preparation technique that yields vesicles appropriate for the intended downstream experimentation.

7.6. Dual-Perfusion of the placenta

Although *in vitro* dual-perfusion of the placenta is not a technique directly related to biobanking, this procedure enables the study of aspects of placental function, including nutrient and drug transport and metabolism [149], transfer of nanoparticles [150], antibodies and viruses [151], synthesis and release of steroid and peptide hormones [152], angiogenic factors, growth factors and cytokines, release of trophoblast microvesicles [153], and vascular reactivity [154]. As the majority of these functions depend on the state of placental metabolism and energy, the conditions necessary for collection and handling of the placenta prior to perfusion are critical and similar to those described previously for collection of placental tissues. In addition, the effects of the perfusion process itself on placental structure and metabolism need to be considered.

Many studies show no differences in functional parameters between placentas delivered vaginally or by caesarean section [155]. This may not be surprising if the caesarean delivered tissue had not been promptly cannulated and had suffered loss of energy charge to the same extent as that from a vaginal delivery. Only utilising placental tissues that are collected quickly following caesarean section in the absence of labour and immediately starting perfusion may address the issue of loss of energy charge. Care should be exercised in removal of the placenta at delivery to avoid tearing of tissue that would lead to leaks during perfusion. The location of the perfusion laboratory immediately adjacent to the delivery facility, and immediate transport from the delivery room, is therefore critically important.

Optimal conditions would be elective caesarean delivery and cannulation of the fetal side of the placenta (most easy to achieve) within 10 min of delivery followed subsequently by cannulation and perfusion of the intervillous space in the next 10 min. The energy charge will drop rapidly after delivery, but can be arrested by cannulation and oxygenation, and, although it will not reach the *in vivo* level, it will stabilise [156], with oxygen and glucose consumption, lactate production and maternal-to-fetal amino acid concentration gradients similar to those in the *in vivo* state.

The adequacy and integrity of perfusion should be monitored during and after the experiment using simple measures, such as constancy of perfusion pressure or of oxygen consumption, minimal loss of perfusate through leakage or transfer between the circuits, or by detailed electron microscopic examination. The success rate claimed varies for 30–75%, being dependent on the rigour of criteria used and outcomes studied. The obvious failures are due to pressure mismatches, leaks or tears in tissue.

As the placenta has a high metabolic rate, it should be perfused with glucose and amino acids (usually a tissue culture medium) rather than a balanced salt solution. This requirement assumes even greater importance if long-term perfusions are the objective [151]. Inclusion of oncotic agents [151] or blood [157] into the

perfusion medium has been shown to prevent swelling of mitochondria and endoplasmic reticulum, oedema and vacuolation in trophoblast during the perfusion [158]. Antibiotics prevent bacterial growth [151] in the media over a prolonged period of perfusion at 37 °C, but gentamycin should be avoided as it induces endoplasmic reticulum stress. Perhaps the most vital item to address when perfusing the placenta is tissue oxygenation, the placenta being an oxygen conformer with oxygen uptake directly related to oxygen supply [159].

In vitro perfusion poses two challenges for placental tissue. First, the placenta is usually perfused with an aqueous media containing no oxygen carrier equivalent to haemoglobin *in vivo*, and perfusate oxygen tensions are often kept low (8–9% O_2) as this mimics the pO_2 in mixed intervillous blood. However, this exposes the tissue to both anaemic hypoxia and hypoxaemic hypoxia. These can be alleviated by either perfusing with aqueous media equilibrated with 95% O_2 using a membrane oxygenator, which increases placental metabolism as evidenced by increased glucose consumption and lactate production [151], but potentially exposes the tissue to hyperoxia and oxygen toxicity [151]. Second, a perfusate containing natural (cord blood or expired blood bank) or synthetic haemoglobin as an oxygen carrier may be used. More recently, perfusion with low oxygen tensions 15% O_2, (5–7% [O_2]) but with many cannulae ($n = 22$) in the intervillous space of a single lobule [160] reportedly overcomes issues of inadequate oxygenation.

8. Ethical issues surrounding collection of placental samples

Any research involving human subjects submitted to a scientific journal needs to follow the principles of the Helsinki Declaration (issued by the The World Medical Association: http://www.wma.net/en/20activities/10ethics/10helsinki/index.html), according to the International Committee of Medical Journal Editors (ICMJE, also named "The Vancouver Group": http://www.icmje.org/).

ICMJE recommends that, when reporting experiments on human subjects, authors should indicate whether the procedures were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki declaration. Fundamental requirements in the Helsinki declaration include that participation must be voluntary and based on adequate information, and that the privacy of research subjects and confidentiality of their personal information are respected. The Helsinki declaration recommends that "if the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed". The Helsinki declaration also states that "For medical research using identifiable human material or data, physicians must normally seek consent for the collection, analysis, storage and/or reuse. There may be situations where consent would be impossible or impractical to obtain for such research or would pose a threat to the validity of the research. In such situations the research may be done only after consideration and approval of a research ethics committee".

Although the placenta may be regarded by some as clinical waste, for others it can be the object of deeply held cultural beliefs [161]. Researchers must therefore be cogniscent of, and sympathetic towards, local values and traditions. The requirements for informed patient consent to sample or access such biological samples for research purposes (even when anonymous) may vary between countries and institutions, and also depend on the study type. Therefore, when setting up prospective research biobanks that include placental-related specimens linked to clinical datasets, we recommend that the local standard procedure of patient information and informed consent is followed.

Little research has been performed on factors influencing patient consent in placental research, but a recent interview-based study looking at recruitment for *ex vivo* perfusion experiments found that the timing when consent was obtained, and the medical personnel involved were important [162]. Delivery, especially vaginal, is a stressful event and so consent is best gained in advance, and women preferred midwives with whom they were familiar to scientists. More importantly, women who understood the purpose of the research were willing to donate their placentas. This may pose problems for biobanks that aim to support a variety of studies. In addition, as research technologies advance, one hopes that biobanks will be accessible for future studies not anticipated at the time of collection. Hansson et al. argue that both broad consent and consent for future research are valid ethically for biobanks with three caveats; personal information related to the research must be handled securely, donors must have the right to withdraw consent, and every new study must be approved by an ethics-review board [163].

When obtaining consent, it must also be stated clearly whether the data or biological samples can be shared with other researchers, and in which form (anonymous or de-identified: linking a code to an identifier only accessible to for example the researcher obtaining the patient consent), and whether potentially identifiable datasets may be used in collaborations (such as genome sequencing etc). Permission should preferably be sought for use of the samples not only in other centres in the same country, but in any centre carrying out relevant research in the world. Exploitative research of all sorts, including, for example collection in developing countries without appropriate ethical considerations, must obviously be avoided [164].

Researchers sharing biological and/or clinical datasets should also consider the need for Material Transfer Agreements (MTA) when involved in research collaboration between institutions and countries. Many institutions have a standard MTA, which describes the nature of the collaboration, discusses intellectual property rights, and usually would include references to legal and institutional approval of the research study. An MTA should usually describe what the recipient of the dataset or biological samples will do with the material when the research project period is over.

9. Collection protocol

We outline a suggested protocol for the collection of placental samples below. This is a comprehensive list, arranged in order of priority of timing based on the evidence presented above, summarised in Table 1. We recommend that the actions highlighted in bold form a basic protocol, and that researchers select additional samples depending on the questions being addressed and the resources available for collection and storage. These actions can be built into local Standard Operating Procedures. Time is of the essence, and it is essential to be prepared in advance. It is also important to record the time elapsed between delivery and final freezing or fixation of the various samples taken, so that this may be taken into consideration during subsequent analyses. A scalpel or disposable brain knife is best for taking the larger full-thickness samples, whereas scissors are best for the smaller ones.

- **Photograph the placenta from the chorionic and basal aspects against a scale bar**
- Sample cord bloods
- **Take a membrane roll 2 cm wide from the rupture site to the placental margin**
- **Trim the membranes and umbilical cord to 1 cm, tying off the cord in the process, and weigh the placenta**
- **Place the placenta with the basal plate uppermost, and overlay a transparent grid identifying at least 4 sampling sites (Fig. 1). At each site remove the basal plate by trimming with a pair of scissors. Then cut out a grape-sized (1–2 cm^3) piece of the exposed villous tissue, avoiding areas of frank**

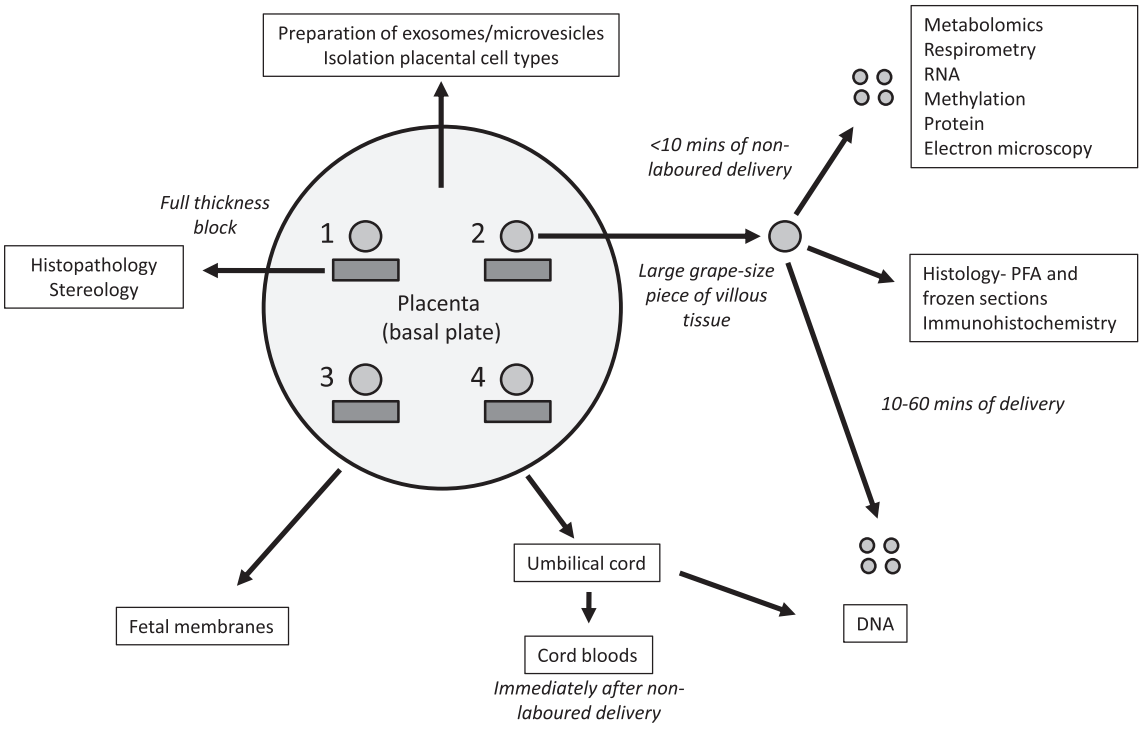

Fig. 1. Schematic representation of placental sampling (adapted from Fig. 1 in Pasupathy et al. [165] with permission).

pathology. Wash thoroughly but gently in PBS at 4 °C. Quickly divide into pieces for:

- Metabolomics: 100 mm^3 (100 mg) snap frozen
- Mitochondrial respirometry: 10 mm^3 (10 mg) snap frozen in cryopreservative medium [127]
- Electron microscopy: for TEM 1–2 mm^3, and for SEM up to 5 mm^3, immersed in 2% glutaraldehyde and 2% formaldehyde at 4 °C for up to 12 h
- **RNA: 5 mm^3 (5 mg) immersed in 1 ml RNAlater for 48 h at 4 °C and then snap frozen**
- **Protein and DNA: 50 mm^3 (50 mg) pieces snap frozen**
- **Immunohistochemistry: pea-size (0.5 cm^3) sample in 5–10 ml aliquot of 4% PFA at 4 °C for 12 h**
- Frozen sections: pea-size (0.5 cm^3) sample immersed in OCT and snap-frozen

Several pieces (at least 4) should be taken from each sample site for each assay, allowing for repeat experiments and/or distribution. For frozen samples the pieces may be spaced around the inner wall of a cryo-vial, allowing individual pieces to be removed without crushing or thawing. For RNAlater or fixative, the volume of fluid should be at least 10× that of the tissue to ensure adequate quantities of reagents.

- **Adjacent to each sample site take a full thickness sample 2 × 1 cm irrespective of frank pathology for histopathology or stereology. Immerse in 10% buffered formalin for 12–24 h**
- Use remaining tissues for exosome/microvesicle preparation, isolation of placental cells
- **Sample umbilical cord. Snap freeze a 100 mg sample for DNA, and immerse a 5 mm section transverse block in 10% buffered formalin for 12–24 h for histology**
- Placental bed/decidua sampling if a caesarean delivery
- Uterine venous blood sampling (20–30 ml) if a caesarean delivery
- **Take maternal blood or saliva for DNA analysis**
- **Complete the placenta biobank with relevant clinical information, including maternal and fetal information**

10. Conclusion

Careful attention to sample collection, processing and storage are critical in order to reduce the number of confounding variables that can influence data derived from the human placenta. This review considers the important factors that we have experienced during the course of our research. Nonetheless, we recognise that there will be other considerations, specific to specialised techniques. Standardisation of sample collection can improve the quality of placental research, and facilitate sharing of samples between groups, allowing larger datasets to be generated. Equally, we appreciate that not all samples can be collected under optimal conditions, but an awareness of the potential confounders can assist researchers in the interpretation of their resultant data.

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References

[1] Perry IJ, Beevers DG, Whincup PH, Bareford D. Predictors of ratio of placental weight to fetal weight in multiethnic community. BMJ 1995;310:436–9.
[2] Mayhew TM, Burton GJ. Stereology and its impact on our understanding of human placental functional morphology. Microsc Res Tech 1997;38:195–205.
[3] Bush PG, Mayhew TM, Abramovich DR, Aggett PJ, Burke MD, Page KR. A quantitative study on the effects of maternal smoking on placental morphology and cadmium concentration. Placenta 2000;21:247–56.
[4] Burd L, Roberts D, Olson M, Odendaal H. Ethanol and the placenta: a review. J Matern Fetal Neonatal Med 2007;20:361–75.

[5] Higgins L, Greenwood SL, Wareing M, Sibley CP, Mills TA. Obesity and the placenta: a consideration of nutrient exchange mechanisms in relation to aberrant fetal growth. Placenta 2011;32:1–7.
[6] Aye IL, Powell TL, Jansson T. Review: adiponectin – the missing link between maternal adiposity, placental transport and fetal growth? Placenta 2013;34S: S40–5.
[7] Roberts VH, Smith J, McLea SA, Heizer AB, Richardson JL, Myatt L. Effect of increasing maternal body mass index on oxidative and nitrative stress in the human placenta. Placenta 2009;30:169–75.
[8] Nomura Y, Lambertini L, Rialdi A, Lee M, Mystal EY, Grabie M, et al. Global methylation in the placenta and umbilical cord blood from pregnancies with maternal gestational diabetes, preeclampsia, and obesity. Reprod Sci 2013 [E-Pub].
[9] Haavaldsen C, Samuelsen SO, Eskild A. The association of maternal age with placental weight: a population-based study of 536,954 pregnancies. BJOG 2011;118:1470–6.
[10] Strom-Roum EM, Haavaldsen C, Tanbo TG, Eskild A. Paternal age, placental weight and placental to birthweight ratio: a population-based study of 590 835 pregnancies. Hum Reprod 2013;28:3126–33.
[11] Wallace JM, Bhattacharya S, Horgan GW. Gestational age, gender and parity specific centile charts for placental weight for singleton deliveries in Aberdeen, UK. Placenta 2013;34:269–74.
[12] Nelson DM, Burton GJ. A technical note to improve the reporting of studies of the human placenta. Placenta 2011;32:195–6.
[13] Mayhew TM, Jenkins H, Todd B, Clifton VL. Maternal asthma and placental morphometry: effects of severity, treatment and fetal sex. Placenta 2008;29: 366–73.
[14] Brass E, Hanson E, O'Tierney-Ginn PF. Placental oleic acid uptake is lower in male offspring of obese women. Placenta 2013;34:503–9.
[15] Clifton VL. Review: sex and the human placenta: mediating differential strategies of fetal growth and survival. Placenta 2010;31(Suppl.):S33–9.
[16] Muralimanoharan S, Maloyan A, Myatt L. Evidence of sexual dimorphism in the placental function with severe preeclampsia. Placenta 2013;34: 1183–9.
[17] Sood R, Zehnder JL, Druzin ML, Brown PO. Gene expression patterns in human placenta. Proc Natl Acad Sci U S A 2006;103:5478–83.
[18] Scott NM, Hodyl NA, Murphy VE, Osei-Kumah A, Wyper H, Hodgson DM, et al. Placental cytokine expression covaries with maternal asthma severity and fetal sex. J Immunol 2009;182:1411–20.
[19] Osei-Kumah A, Smith R, Jurisica I, Caniggia I, Clifton VL. Sex-specific differences in placental global gene expression in pregnancies complicated by asthma. Placenta 2011;32:570–8.
[20] Mao J, Zhang X, Sieli PT, Falduto MT, Torres KE, Rosenfeld CS. Contrasting effects of different maternal diets on sexually dimorphic gene expression in the murine placenta. Proc Natl Acad Sci U S A 2010;107:5557–62.
[21] Yeganegi M, Leung CG, Martins A, Kim SO, Reid G, Challis JR, et al. *Lactobacillus rhamnosus* GR-1 stimulates colony-stimulating factor 3 (granulocyte) (CSF3) output in placental trophoblast cells in a fetal sex-dependent manner. Biol Reprod 2011;84:18–25.
[22] Brar HS, Platt LD, DeVore GR, Horenstein J, Medearis AL. Qualitative assessment of maternal uterine and fetal umbilical artery blood flow and resistance in laboring patients by Doppler velocimetry. Am J Obstet Gynecol 1988;158: 952–6.
[23] Ramsey EM, Donner MW. Placental vasculature and circulation. Anatomy, physiology, radiology, clinical aspects, atlas and textbook. Stuttgart: Georg Thieme; 1980. p. 101.
[24] Fleischer A, Anyaegbunam AA, Schulman H, Farmakides G, Randolph G. Uterine and umbilical artery velocimetry during normal labor. Am J Obstet Gynecol 1987;157:40–3.
[25] Fratelli N, Prefumo F, Andrico S, Lorandi A, Recupero D, Tomasoni G, et al. Effects of epidural analgesia on uterine artery Doppler in labour. Br J Anaesth 2011;106:221–4.
[26] Sorensen A, Peters D, Simonsen C, Pedersen M, Stausbol-Gron B, Christiansen OB, et al. Changes in human fetal oxygenation during maternal hyperoxia as estimated by BOLD MRI. Prenat Diagn 2013;33:141–5.
[27] Burton GJ, Ingram SC, Palmer ME. The influence of the mode of fixation on morphometrical data derived from terminal villi in the human placenta at term: a comparison of immersion and perfusion fixation. Placenta 1987;8: 37–51.
[28] Mayhew TM, Sorensen FB, Klebe JG, Jackson MR. The effects of mode of delivery and sex of newborn on placental morphology in control and diabetic pregnancies. J Anat 1993;183(Pt 3):545–52.
[29] Bouw GM, Stolte LAM, Baak JPA, Oort J. Quantitative morphology of the placenta. I. Standardization of sampling. Eur J Obstet Gynecol Reprod Biol 1976;6:325–31.
[30] Farrar D, Airey R, Law GR, Tuffnell D, Cattle B, Duley L. Measuring placental transfusion for term births: weighing babies with cord intact. BJOG 2011;118:70–5.
[31] Bloxam DL, Bobinski PM. Energy metabolism and glycolysis in the human placenta during ischaemia and in normal labour. Placenta 1984;5:381–94.
[32] Mendez-Figueroa H, Chien EK, Ji H, Nesbitt NL, Bharathi SS, Goetzman E. Effects of labor on placental fatty acid beta oxidation. J Matern Fetal Neonatal Med 2013;26:150–4.
[33] Tissot van Patot MC, Murray AJ, Beckey V, Cindrova-Davies T, Johns J, Zwerdlinger L, et al. Human placental metabolic adaptation to chronic hypoxia, high altitude: hypoxic preconditioning. Am J Physiol Regul Integr Comp Physiol 2010;298:R166–72.
[34] Many A, Roberts JM. Increased xanthine oxidase during labour-implications for oxidative stress. Placenta 1997;18:725–6.
[35] Cindrova-Davies T, Yung HW, Johns J, Spasic-Boskovic O, Korolchuk S, Jauniaux E, et al. Oxidative stress, gene expression, and protein changes induced in the human placenta during labor. Am J Pathol 2007;171: 1168–79.
[36] Cindrova-Davies T. From placental oxidative stress to maternal endothelial dysfunction. Placenta Suppl A 2009;30:S55–65.
[37] Abdulsid A, Hanretty K, Lyall F. Heat shock protein 70 expression is spatially distributed in human placenta and selectively upregulated during labor and preeclampsia. PLoS One 2013;8:e54540.
[38] Roland L, Beauchemin D, Acteau G, Fradette C, St-Pierre I, Bilodeau JF. Effects of labor on placental expression of superoxide dismutases in preeclampsia. Placenta 2010;31:392–400.
[39] Sitras V, Paulssen RH, Gronaas H, Vartun A, Acharya G. Gene expression profile in labouring and non-labouring human placenta near term. Mol Hum Reprod 2008;14:61–5.
[40] Lee KJ, Shim SH, Kang KM, Kang JH, Park DY, Kim SH, et al. Global gene expression changes induced in the human placenta during labor. Placenta 2010;31:698–704.
[41] Peng HH, Kao CC, Chang SD, Chao AS, Chang YL, Wang CN, et al. The effects of labor on differential gene expression in parturient women, placentas, and fetuses at term pregnancy. Kaohsiung J Med Sci 2011;27:494–502.
[42] Rodriguez-Prado YM, Kong X, Fant ME. PLAC1 expression decreases in chorionic villi in response to labor. ISRN Obstet Gynecol 2013;2013:704252.
[43] Melvin A, Rocha S. Chromatin as an oxygen sensor and active player in the hypoxia response. Cell Signal 2012;24:35–43.
[44] Schlinzig T, Johansson S, Gunnar A, Ekstrom TJ, Norman M. Epigenetic modulation at birth – altered DNA-methylation in white blood cells after caesarean section. Acta Paediatr 2009;98:1096–9.
[45] Burton GJ, Jones CJ. Syncytial knots, sprouts, apoptosis, and trophoblast deportation from the human placenta. Taiwan J Obstet Gynecol 2009;48: 28–37.
[46] Askelund KJ, Chamley LW. Trophoblast deportation part I: review of the evidence demonstrating trophoblast shedding and deportation during human pregnancy. Placenta 2011;32:716–23.
[47] Germain SJ, Sacks GP, Sooranna SR, Sargent IL, Redman CW. Systemic inflammatory priming in normal pregnancy and preeclampsia: the role of circulating syncytiotrophoblast microparticles. J Immunol 2007;178:5949–56.
[48] Reddy A, Zhong XY, Rusterholz C, Hahn S, Holzgreve W, Redman CW, et al. The effect of labour and placental separation on the shedding of syncytiotrophoblast microparticles, cell-free DNA and mRNA in normal pregnancy and pre-eclampsia. Placenta 2008;29:942–9.
[49] Yano Y, Shiba E, Kambayashi J, Sakon M, Kawasaki T, Fujitani K, et al. The effects of calpeptin (a calpain specific inhibitor) on agonist induced microparticle formation from the platelet plasma membrane. Thromb Res 1993;71:385–96.
[50] Ahn YS, Jy W, Jimenez JJ, Horstman LL. More on: cellular microparticles: what are they bad or good for? J Thromb Haemost 2004;2:1215–6.
[51] Thery C, Zitvogel L, Amigorena S. Exosomes: composition, biogenesis and function. Nat Rev Immunol 2002;2:569–79.
[52] Pantham P, Askelund KJ, Chamley LW. Trophoblast deportation part II: a review of the maternal consequences of trophoblast deportation. Placenta 2011;32:724–31.
[53] Abumaree MH, Chamley LW, Badri M, El-Muzaini MF. Trophoblast debris modulates the expression of immune proteins in macrophages: a key to maternal tolerance of the fetal allograft? J Reprod Immunol 2012;94: 131–41.
[54] Lemtis H, Hadrich G. On the decrease of the placental weight following delivery and its implication for the relationship between placental and neonatal weight (author's transl). Geburtshilfe Frauenheilkd 1974;34: 618–22.
[55] Fox H, Sebire NJ. Pathology of the placenta. Amsterdam: Saunders Elsevier; 2007. p. 574.
[56] Benirschke K, Burton GJ, Baergen RN. Pathology of the human placenta. Heidelberg: Springer; 2012. p. 941.
[57] Kaufmann P. Influence of ischemia and artificial perfusion on placental ultrastructure and morphometry. Contrib Gynecol Obstet 1985;13:18–26.
[58] Yung H, Colleoni F, Atkinson D, Cook E, Murray AJ, Burton GJ, et al. Influence of speed of sample processing on placental energetics and signalling pathways; implications for tissue collection. Placenta 2013 [in press].
[59] Serkova N, Bendrick-Peart J, Alexander B, Tissot van Patot MC. Metabolite concentrations in human term placentae and their changes due to delayed collection after delivery. Placenta 2003;24:227–35.
[60] Liu L, Wise DR, Diehl JA, Simon MC. Hypoxic reactive oxygen species regulate the integrated stress response and cell survival. J Biol Chem 2008;283: 31153–62.
[61] Fahling M. Surviving hypoxia by modulation of mRNA translation rate. J Cell Mol Med 2009;13:2770–9.
[62] Feneley MR, Burton GJ. Villous composition and membrane thickness in the human placenta at term: a stereological study using unbiased estimators and optimal fixation techniques. Placenta 1991;12:131–42.

[63] Burton GJ. The fine structure of the human placenta as revealed by scanning electron microscopy. Scanning Microsc 1987;1:1811–28.
[64] Garrod A, Batra G, Ptacek I, Heazell AEP. Duration and method of tissue storage alters placental morphology – implications for clinical and research practice. Placenta 2013;34:1116–9.
[65] Leary SD, Godfrey KM, Greenaway LJ, Davill VA, Fall CH. Contribution of the umbilical cord and membranes to untrimmed placental weight. Placenta 2003;24:276–8.
[66] Teng J, Chang T, Reyes C, Nelson KB. Placental weight and neurologic outcome in the infant: a review. J Matern Fetal Neonatal Med 2012;25:2082–7.
[67] Coall DA, Charles AK, Salafia CM. Gross placental structure in a low-risk population of singleton, term, first-born infants. Pediatr Dev Pathol 2009;12:200–10.
[68] Pathak S, Hook E, Hackett G, Murdoch E, Sebire NJ, Jessop F, et al. Cord coiling, umbilical cord insertion and placental shape in an unselected cohort delivering at term: relationship with common obstetric outcomes. Placenta 2010;31:963–8.
[69] Pathak S, Sebire NJ, Hook L, Hackett G, Murdoch E, Jessop F, et al. Relationship between placental morphology and histological findings in an unselected population near term. Virchows Arch 2011;459:11–20.
[70] Grbesa D, Durst-Zivkovic B. Neonatal and placental factors in relation to the mode of umbilical cord insertion. Stereological analysis of chorionic villi. Pflugers Arch 1996;431:R205–6.
[71] Wyatt SM, Kraus FT, Roh CR, Elchalal U, Nelson DM, Sadovsky Y. The correlation between sampling site and gene expression in the term human placenta. Placenta 2005;26:372–9.
[72] Salafia CM, Yampolsky M, Misra DP, Shlakhter O, Haas D, Eucker B, et al. Placental surface shape, function, and effects of maternal and fetal vascular pathology. Placenta 2010;31:958–62.
[73] Yampolsky M, Salafia CM, Shlakhter O, Haas D, Eucker B, Thorp J. Modeling the variability of shapes of a human placenta. Placenta 2008;29:790–7.
[74] Yampolsky M, Salafia CM, Shlakhter O, Haas D, Eucker B, Thorp J. Centrality of the umbilical cord insertion in a human placenta influences the placental efficiency. Placenta 2009;30:1058–64.
[75] Burton GJ, Jauniaux E, Charnock-Jones DS. The influence of the intrauterine environment on human placental development. Int J Dev Biol 2010;54:303–12.
[76] Winder NR, Krishnaveni GV, Veena SR, Hill JC, Karat CL, Thornburg KL, et al. Mother's lifetime nutrition and the size, shape and efficiency of the placenta. Placenta 2011;32:806–10.
[77] Alwasel SH, Abotalib Z, Aljarallah JS, Osmond C, Alkharaz SM, Alhazza IM, et al. Secular increase in placental weight in Saudi Arabia. Placenta 2011;32:391–4.
[78] Barker DJ, Osmond C, Thornburg KL, Kajantie E, Eriksson JG. The lifespan of men and the shape of their placental surface at birth. Placenta 2011;32:783–7.
[79] Barker DJ, Osmond C, Thornburg KL, Kajantie E, Eriksson JG. The shape of the placental surface at birth and colorectal cancer in later life. Am J Hum Biol 2013;25:566–8.
[80] Barker D, Osmond C, Grant S, Thornburg K, Cooper C, Ring S, et al. Maternal cotyledons at birth predict blood pressure in childhood. Placenta 2013;34:672–5.
[81] Hillier SL, Krohn MA, Kiviat NB, Watts DH, Eschenbach DA. Microbiologic causes and neonatal outcomes associated with chorioamnion infection. Am J Obstet Gynecol 1991;165:955–61.
[82] Dong Y, St Clair PJ, Ramzy I, Kagan-Hallet KS, Gibbs RS. A microbiologic and clinical study of placental inflammation at term. Obstet Gynecol 1987;70:175–82.
[83] Pankuch GA, Appelbaum PC, Lorenz RP, Botti JJ, Schachter J, Naeye RL. Placental microbiology and histology and the pathogenesis of chorioamnionitis. Obstet Gynecol 1984;64:802–6.
[84] Aquino TI, Zhang J, Kraus FT, Knefel R, Taff T. Subchorionic fibrin cultures for bacteriologic study of the placenta. Am J Clin Pathol 1984;81:482–6.
[85] Queiros da Mota V, Prodhom G, Yan P, Hohlfheld P, Greub G, Rouleau C. Correlation between placental bacterial culture results and histological chorioamnionitis: a prospective study on 376 placentas. J Clin Pathol 2013;66:243–8.
[86] Farraj AA. Randomized placental and cord blood sampling culture in women with preterm and term labour to detect infection. East Mediterr Health J 2000;6:272–5.
[87] Mayhew TM, Burton GJ. Methodological problems in placental morphometry: apologia for the use of stereology based on sound sampling practice. Placenta 1988;9:565–81.
[88] Mayhew TM. Taking tissue samples from the placenta: an illustration of principles and strategies. Placenta 2008;29:1–14.
[89] Zhang P, Schmidt M, Cook L. Maternal vasculopathy and histologic diagnosis of preeclampsia: poor correlation of histologic changes and clinical manifestation. Am J Obstet Gynecol 2006;194:1050–6.
[90] Driscoll SG, Langston C. College of American pathologists conference XIX on the examination of the placenta: report of the working group on methods for placental examination. Arch Pathol Lab Med 1991;115:704–8.
[91] Langston C, Kaplan C, Macpherson T, Manci E, Peevy K, Clark B, et al. Practice guideline for examination of the placenta: developed by the Placental Pathology Practice Guideline Development Task Force of the College of American Pathologists. Arch Pathol Lab Med 1997;121:449–76.
[92] Hargitai B, Marton T, Cox PM. Best practice no 178. Examination of the human placenta. J Clin Pathol 2004;57:785–92.
[93] Booth VJ, Nelson KB, Dambrosia JM, Grether JK. What factors influence whether placentas are submitted for pathologic examination? Am J Obstet Gynecol 1997;176:567–71.
[94] Spencer MK, Khong TY. Conformity to guidelines for pathologic examination of the placenta. Arch Pathol Lab Med 2003;127:205–7.
[95] Wigglesworth JS. Vascular anatomy of the human placenta and its significance for placental pathology. J Obstet Gynaecol Br Commonw 1969;76:979–89.
[96] Hempstock J, Bao Y-P, Bar-Issac M, Segaren N, Watson AL, Charnock Jones DS, et al. Intralobular differences in antioxidant enzyme expression and activity reflect oxygen gradients within the human placenta. Placenta 2003;24:517–23.
[97] Fox H. The pattern of villous variability in the normal placenta. J Obstet Gynaecol Br Commonw 1964;71:749–58.
[98] Schuhmann RA. Placentone structure of the human placenta. Bibl Anat 1982;22:46–57.
[99] Sala MA, Matheus M. Regional variation of the vasculo-syncytial membranes in the human full-term placenta. Gegenbaurs Morphol Jahrb 1986;132:285–9.
[100] Critchley GR, Burton GJ. Intralobular variations in barrier thickness in the mature human placenta. Placenta 1987;8:185–94.
[101] Schuhmann R, Stoz F, Maier M. Histometric investigations in placentones (materno-fetal circulation units) of human placentae. Troph Res 1988;3:3–16.
[102] Alvarez H, Benedetti WL, Morel RL, Scavarelli M. Trophoblast development gradient and its relationship to placental hemodynamics. Am J Obstet Gynecol 1970;106:416–20.
[103] Brameld JM, Hold R, Pipkin FB. Regional variation in angiotensin converting enzyme activity in the human placenta. Placenta 2011;32:906–8.
[104] Tzschoppe AA, Struwe E, Dorr HG, Goecke TW, Beckmann MW, Schild RL, et al. Differences in gene expression dependent on sampling site in placental tissue of fetuses with intrauterine growth restriction. Placenta 2011;31:178–85.
[105] Jacques SM, Qureshi F, Johnson A, Alkatib AA, Kmak DC. Estimation of time of fetal death in the second trimester by placental histopathological examination. Pediatr Dev Pathol 2003;6:226–32.
[106] Kim YW, Kim JS, Lee KA, Shim JY, Won HS, Lee PR, et al. Memb-roller: an effective way of making membrane rolls for pathological examination and studies of human placenta. Placenta 2013;34:722–5.
[107] Sebire NJ, Goldin RD. Distribution of histological chorioamnionitis in placental membranes; does a membrane roll provide additional information? J Pathol 2001;195:18a.
[108] Walford N, Htun K, Akhilesh M. Detection of atherosis in preeclamptic placentas: comparison of two gross sampling protocols. Pediatr Dev Pathol 2005;8:61–5.
[109] Winters R, Waters BL. What is adequate sampling of extraplacental membranes?: a randomized, prospective analysis. Arch Pathol Lab Med 2008;132:1920–3.
[110] Pijnenborg R, Brosens I, Romero R, editors. Placental bed disorders. Basic science and its translation to obstetrics. Cambridge: Cambridge University Press; 2010.
[111] Brosens I, Khong TY. Defective spiral artery remodeling. In: Pijnenborg R, Brosens I, Romero R, editors. Placental bed disorders. Basic science and its translation to obstetrics. Cambridge: Cambridge University Press; 2010.
[112] Staff AC, Ranheim T, Khoury J, Henriksen T. Increased contents of phospholipids, cholesterol, and lipid peroxides in decidua basalis in women with preeclampsia. Am J Obstet Gynecol 1999;180:587–92.
[113] Staff AC, Halvorsen B, Ranheim T, Henriksen T. Elevated level of free 8-iso-prostaglandin F2alpha in the decidua basalis of women with preeclampsia. Am J Obstet Gynecol 1999;181:1211–5.
[114] Harsem NK, Staff AC, He L, Roald B. The decidual suction method: a new way of collecting decidual tissue for functional and morphological studies. Acta Obstet Gynecol Scand 2004;83:724–30.
[115] Cronin MT, Dutta D, Pho M, Nguyen A, Jeong J, Liu ML. Tumor marker discovery by expression profiling RNA from formalin fixed paraffin embedded tissues. Methods Mol Biol 2009;520:177–93.
[116] Bhatti P, Kampa D, Alexander BH, McClure C, Ringer D, Doody MM, et al. Blood spots as an alternative to whole blood collection and the effect of a small monetary incentive to increase participation in genetic association studies. BMC Med Res Methodol 2009;9:76.
[117] Abraham JE, Maranian MJ, Spiteri I, Russell R, Ingle S, Luccarini C, et al. Saliva samples are a viable alternative to blood samples as a source of DNA for high throughput genotyping. BMC Med Genomics 2012;5:19.
[118] Matthews AM, Kaur H, Dodd M, D'Souza J, Liloglou T, Shaw RJ, et al. Saliva collection methods for DNA biomarker analysis in oral cancer patients. Br J Oral Maxillofac Surg 2013;51:394–8.
[119] Ng DP, Koh D, Choo SG, Ng V, Fu Q. Effect of storage conditions on the extraction of PCR-quality genomic DNA from saliva. Clin Chim Acta 2004;343:191–4.
[120] Rajakumar A, Cerdeira AS, Rana S, Zsengeller Z, Edmunds L, Jeyabalan A, et al. Transcriptionally active syncytial aggregates in the maternal circulation may contribute to circulating soluble fms-like tyrosine kinase 1 in preeclampsia. Hypertension 2012;59:256–64.

[121] Rajakumar A, Michael HM, Rajakumar PA, Shibata E, Hubel CA, Karumanchi SA, et al. Extra-placental expression of vascular endothelial growth factor receptor-1, (Flt-1) and soluble Flt-1 (sFlt-1), by peripheral blood mononuclear cells (PBMCs) in normotensive and preeclamptic pregnant women. Placenta 2005;26:563–73.
[122] Akane H, Saito F, Yamanaka H, Shiraki A, Imatanaka N, Akahori Y, et al. Methacarn as a whole brain fixative for gene and protein expression analyses of specific brain regions in rats. J Toxicol Sci 2013;38:431–43.
[123] San Martin S, Fitzgerald JS, Weber M, Parraga M, Saez T, Zorn TM, et al. Stat3 and Socs3 expression patterns during murine placenta development. Eur J Histochem 2013;57:e19.
[124] Skepper JN. Immunocytochemical strategies for electron microscopy: choice or compromise. J Microsc 2000;199:1–36.
[125] Heazell AE, Brown M, Dunn WB, Worton SA, Crocker IP, Baker PN, et al. Analysis of the metabolic footprint and tissue metabolome of placental villous explants cultured at different oxygen tensions reveals novel redox biomarkers. Placenta 2008;29:691–8.
[126] Kuznetsov AV, Veksler V, Gellerich FN, Saks V, Margreiter R, Kunz WS. Analysis of mitochondrial function in situ in permeabilized muscle fibers, tissues and cells. Nat Protoc 2008;3:965–76.
[127] Colleoni F, Morash AJ, Ashmore T, Monk M, Burton GJ, Murray AJ. Cryopreservation of placental biopsies for mitochondrial respiratory analysis. Placenta 2012;33:122–3.
[128] Mayhew TM, Huppertz B, Kaufmann P, Kingdom JC. The 'reference trap' revisited: examples of the dangers in using ratios to describe fetoplacental angiogenesis and trophoblast turnover. Placenta 2003;24:1–7.
[129] Laga EM, Driscoll SG, Munro HN. Quantitative studies of human placenta. I. Morphometry. Biol Neonate 1973;23:231–59.
[130] Burton GJ, Jauniaux E. Sonographic, stereological and Doppler flow velocimetric assessments of placental maturity. Br J Obstet Gynaecol 1995;102:818–25.
[131] Howard C, Reed MG. Unbiased stereology. Three dimensional measurement in microscopy. Liverpool: QTP Publications; 2005. p. 278.
[132] Mattfeldt T, Mall G, Gharehbaghi H. Estimation of surface area and length with the orientator. J Microsc 1990;159:301–17.
[133] Mayhew TM. Stereology and the placenta: where's the point? – a review. Placenta 2006;27(Suppl. A):S17–25.
[134] Kliman HJ, Nestler JE, Sermasi E, Sanger JM, Strauss 3rd JF. Purification, characterization, and in vitro differentiation of cytotrophoblasts from human term placentae. Endocrinology 1986;118:1567–82.
[135] Trundley A, Gardner L, Northfield J, Chang C, Moffett A. Methods for isolation of cells from the human fetal-maternal interface. Methods Mol Med 2006;122:109–22.
[136] Ilic D, Kapidzic M, Genbacev O. Isolation of human placental fibroblasts. Curr Protoc Stem Cell Biol 2008;5. 1c.6.1–1c.6.17.
[137] Tang Z, Tadesse S, Norwitz E, Mor G, Abrahams VM, Guller S. Isolation of Hofbauer cells from human term placentas with high yield and purity. Am J Reprod Immunol 2011;66:336–48.
[138] Male V, Gardner L, Moffett A. Isolation of cells from the feto-maternal interface. Curr Protoc Immunol 2012;40:1–11 [Chapter 7: Unit 7].
[139] Cindrova-Davies T, Spasic-Boskovic O, Jauniaux E, Charnock-Jones DS, Burton GJ. Nuclear factor-kappa B, p38, and stress-activated protein kinase mitogen-activated protein kinase signaling pathways regulate proinflammatory cytokines and apoptosis in human placental explants in response to oxidative stress: effects of antioxidant vitamins. Am J Pathol 2007;170:1511–20.
[140] Abumaree MH, Stone PR, Chamley LW. An in vitro model of human placental trophoblast deportation/shedding. Mol Hum Reprod 2006;12:687–94.
[141] Siman CM, Sibley CP, Jones CJ, Turner MA, Greenwood SL. The functional regeneration of syncytiotrophoblast in cultured explants of term placenta. Am J Physiol Regul Integr Comp Physiol 2001;280:R1116–22.
[142] Gupta AK, Rusterholz C, Huppertz B, Malek A, Schneider H, Holzgreve W, et al. A comparative study of the effect of three different syncytiotrophoblast micro-particles preparations on endothelial cells. Placenta 2005;26:59–66.
[143] Gupta AK, Rusterholz C, Holzgreve W, Hahn S. Syncytiotrophoblast microparticles do not induce apoptosis in peripheral T lymphocytes, but differ in their activity depending on the mode of preparation. J Reprod Immunol 2005;68:15–26.
[144] Southcombe J, Tannetta D, Redman C, Sargent I. The immunomodulatory role of syncytiotrophoblast microvesicles. PLoS One 2011;6:e20245.
[145] Smarason AK, Sargent IL, Starkey PM, Redman CW. The effect of placental syncytiotrophoblast microvillous membranes from normal and pre-eclamptic women on the growth of endothelial cells in vitro. Br J Obstet Gynaecol 1993;100:943–9.
[146] Kshirsagar SK, Alam SM, Jasti S, Hodes H, Nauser T, Gilliam M, et al. Immunomodulatory molecules are released from the first trimester and term placenta via exosomes. Placenta 2012;33:982–90.
[147] Tannetta DS, Sargent IL, Linton EA, Redman CW. Vitamins C and E inhibit apoptosis of cultured human term placenta trophoblast. Placenta 2008;29:680–90.
[148] Tannetta DS, Dragovic RA, Gardiner C, Redman CW, Sargent IL. Characterisation of syncytiotrophoblast vesicles in normal pregnancy and pre-eclampsia: expression of Flt-1 and endoglin. PLoS One 2013;8:e56754.
[149] Hutson JR, Garcia-Bournissen F, Davis A, Koren G. The human placental perfusion model: a systematic review and development of a model to predict in vivo transfer of therapeutic drugs. Clin Pharmacol Ther 2011;90:67–76.
[150] Wick P, Malek A, Manser P, Meili D, Maeder-Althaus X, Diener L, et al. Barrier capacity of human placenta for nanosized materials. Environ Health Perspect 2010;118:432–6.
[151] Polliotti BM, Holmes R, Cornish JD, Hulsey M, Keesling S, Schwartz D, et al. Long-term dual perfusion of isolated human placental lobules with improved oxygenation for infectious diseases research. Placenta 1996;17:57–68.
[152] Linnemann K, Malek A, Sager R, Blum WF, Schneider H, Fusch C. Leptin production and release in the dually in vitro perfused human placenta. J Clin Endocrinol Metab 2000;85:4298–301.
[153] Guller S, Tang Z, Ma YY, Di Santo S, Sager R, Schneider H. Protein composition of microparticles shed from human placenta during placental perfusion: potential role in angiogenesis and fibrinolysis in preeclampsia. Placenta 2011;32:63–9.
[154] Myatt L. Control of vascular resistance in the human placenta. Placenta 1992;13:329–41.
[155] Mose T, Mathiesen L, Karttunen V, Nielsen JK, Sieppi E, Kummu M, et al. Meta-analysis of data from human ex vivo placental perfusion studies on genotoxic and immunotoxic agents within the integrated European project NewGeneris. Placenta 2012;33:433–9.
[156] Bloxam DL, Bullen BE. Condition and performance of the perfused human placental cotyledon. Am J Obstet Gynecol 1986;155:382–8.
[157] Contractor SF, Eaton BM, Firth JA, Bauman KF. A comparison of the effects of different perfusion regimes on the structure of the isolated human placental lobule. Cell Tissue Res 1984;237:609–17.
[158] Illsley NP, Fox H, Van der Veen F, Chawner L, Penfold P. Human placental ultrastructure after in vitro dual perfusion. Placenta 1985;6:23–32.
[159] Schneider H. Placental oxygen consumption. Part II: in vitro studies-a review. Placenta 2000;21(Suppl. A):S38–44.
[160] Soydemir F, Kuruvilla S, Brown M, Dunn W, Day P, Crocker IP, et al. Adapting in vitro dual perfusion of the human placenta to soluble oxygen tensions associated with normal and pre-eclamptic pregnancy. Lab Invest 2011;91:181–9.
[161] Yoshizawa RS. Review: public perspectives on the utilization of human placentas in scientific research and medicine. Placenta 2013;34:9–13.
[162] Halkoaho A, Pietila AM, Dumez B, Van Damme K, Heinonen S, Vahakangas K. Ethical aspects of human placental perfusion: interview of the mothers donating placenta. Placenta 2010;31:686–90.
[163] Hansson MG, Dillner J, Bartram CR, Carlson JA, Helgesson G. Should donors be allowed to give broad consent to future biobank research? Lancet Oncol 2006;7:266–9.
[164] Upshur RE, Lavery JV, Tindana PO. Taking tissue seriously means taking communities seriously. BMC Med Ethics 2007;8:11.
[165] Pasupathy D, Dacey A, Cook E, Charnock-Jones DS, White IR, Smith GC. Study protocol. A prospective cohort study of unselected primiparous women: the pregnancy outcome prediction study. BMC Pregnancy Childbirth 2008;8:51.