CORRELATION OF MACROSCOPIC AND MICROSCOPIC PLACENTAL LESIONS WITH OBSTETRIC AND NEONATAL OUTCOMES IN AN UNSELECTED POPULATION

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ABSTRACT

Many abnormalities of the placenta are reported to be significant in the setting of maternal health problems and adverse fetal/neonatal outcomes. In specific clinicopathological circumstances - eg vascular lesions in the growth restricted fetus – correlation does exist between the placental lesion and the clinical event. Relationships between "lower grade" placental lesions and clinical outcomes are less clear. Reports of associations between selected abnormalities and clinical outcomes are largely based on retrospective case control studies: the clinical groups studied tend to be high-risk. Understanding of the significance of these lesions in the wider population is lacking.

This study reports on the clinical events and placental lesions documented in 1119 unselected women delivering at the conclusion of a singleton pregnancy in a single obstetric centre. Study methodology was such that the cohort comprised low-risk mothers delivering at or close to term.

The incidence of potentially adverse obstetric and neonatal events in the study population was low. 97% delivered at term. Mean birth weight was 3485 g; mean Apgar scores were 9 at 1 minute and 10 at 5 minutes. 5.9% of infants required admission to neonatal intensive care. When classified in accordance with current standard reporting guidelines, 71% of placentas were classified as normal. Inclusion of lower grade histological lesions in the reporting schedule reduced the percentage of histologically normal placentas to 58%. Funisitis was found to be significantly correlated with adverse neonatal outcome. A number of other placental lesions - including cord coiling <10th and >90th centiles, placental infarction, villitis of unknown etiology and lower grades of acute placental inflammation - were not found to be associated with adverse obstetric or neonatal events. It is concluded that a number of placental lesions may not be relevant to adverse pregnancy outcomes in a low-risk population.

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DEDICATION

To the memory of George T.J. Hamilton, 1933 – 2009**.**

CHAPTER ONE: INTRODUCTION

Summary:

- Brief background with justification of study
- Aims and objectives
- Normal placenta
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    - Macroscopic examination of the placenta
    - Normal macroscopic appearance
    - Normal histology of third trimester placenta
- Definitions of key placental lesions
    - Macroscopic
    - Histological
- Definitions of adverse clinical events
- Current evidence supporting association of adverse clinical events and key placental lesions

1.1 BACKGROUND

Since the 19th century, a fundamental concept of histopathology has been the correlation of reproducibly recognizable abnormal morphological patterns with specific disease processes: Rokitansky's and Aschoff's realization that identifiable macroscopic abnormalities seen at post-mortem examination mapped to an individual disease noted in the patient before death (1) was subsequently developed by Rudolf Virchow, who linked histologically recognizable patterns of cell injury with specific disease processes (2). In modern clinicopathological practice, a specific disease may be considered to be understood fully when the aetiology (cause) and pathogenesis (mechanisms of development) are defined, a morphological pattern recognized and a clinical consequence understood.

For a large number of human diseases, this four step pathway is at least partially complete. As a minimum, in the day to day clinical practice of modern histopathology, the definition and recognition of a morphological pattern must be sufficiently secure to allow a diagnosis to be recorded and a clinical management plan to be instituted, even if a full understanding of the environmental and genetic events underlying the aetiology and pathogenesis is more limited.

The process of disease recognition begun by Virchow's microscopic observations of human tissues many decades ago is, for the placenta, fragmented and lacking in evidence. There exist large numbers of publications concerning a wide spectrum of histological abnormalities of the placenta, but study design is often limited to case series, case control or retrospective cohort analysis. Clinical and histological definitions of specific disease processes in many placental studies are extremely variable (3, 4). Also important, if difficult to quantify, is the effect of progress in medical care. Clinical outcomes are based not only on maternal clinical disease or placental pathology (well characterised or otherwise) but are also highly dependent on clinical care received during the progress of the pregnancy. Historical differences in clinical outcomes between older and more recent studies are likely, making comparison of older and contemporary studies potentially unreliable.

Whether the starting points for review of the literature are macroscopic/microscopic findings projected onto clinical outcomes or clinical outcomes reviewed retrospectively against placental morphology, the findings are confusing, conflicting and difficult to apply

to routine clinical practice. Here, current studies and hypotheses are examined, highlighting where appropriate conflicting evidence and uncertainties.

1.2. AIMS AND OBJECTIVES

This study, which prospectively follows, in a single centre, an unselected cohort of 1119 subjects with an ongoing singleton pregnancy delivering at or close to term, seeks to define the associations of clearly defined placental lesions with specific clinical outcomes in term pregnancy.

Normal growth and development of the placenta is reviewed, emphasizing those aspects which, when disrupted, are currently believed to underly subsequent histological abnormalities and adverse clinical outcomes. A brief overview of selected macroscopic findings in the term placenta (including the umbilical cord) is given. A description of normal term placental histology is presented, together with definitions of abnormal placental histological findings, the latter placed in the context of current published evidence for clinicopathological findings. Current concepts of mechanisms of placental disease are explored. Definitions of significant clinical outcomes are given. A comprehensive review explores the current evidence linking placental abnormality with adverse clinical outcome from two perspectives: prospective studies describing placental abnormality with clinical outcomes and retrospective studies recording placental changes in the setting of defined clinical events.

As an initial step, all delivered placentas in this study are reported histologically within the framework of current normal clinical practice. To determine whether the presence of some rarer placental abnormalities, or the severity or extent of more specifically defined common placental abnormalities correlates well with clinical complications of pregnancy, a more extended morphological and histomorphometric analysis of placental subgroups within the cohort is undertaken and related to detailed clinical outcomes.

The findings from the analysis of umbilical cord coiling are placed in the relatively novel context - for a histopathological study - of systematic reviewing of the relevant literature. The conventional systematic review construct is modified to create a model allowing assessment of the clinical implications of a suspected pathological change.

Pilot immunohistochemical studies are undertaken, with analysis of the inflammatory cell population in presumed non-infective placental inflammation (villitis of unknown etiology) and markers of apoptosis and oxidative stress injury in placental infarction .

1.3. NORMAL PLACENTAL DEVELOPMENT AND HISTOLOGY

1.3.1 Normal placental development

Detailed descriptions of normal placental histology date from the 1950s and are based on a unique collection of embryos identified within hysterectomy specimens, assembled in Cambridge by JD Boyd and studied in collaboration with WJ Hamilton (Charing Cross Medical School). Descriptions of the histological appearances of these embryos, from the earliest days of implantation to crown-rump length (CRL) of approximately 90mm, remain the most comprehensive within the published literature (5, 6). Photomicrographs of haematoxylin and eosin stained whole mount uterine sections from the collection are shown in Figure 1.1.

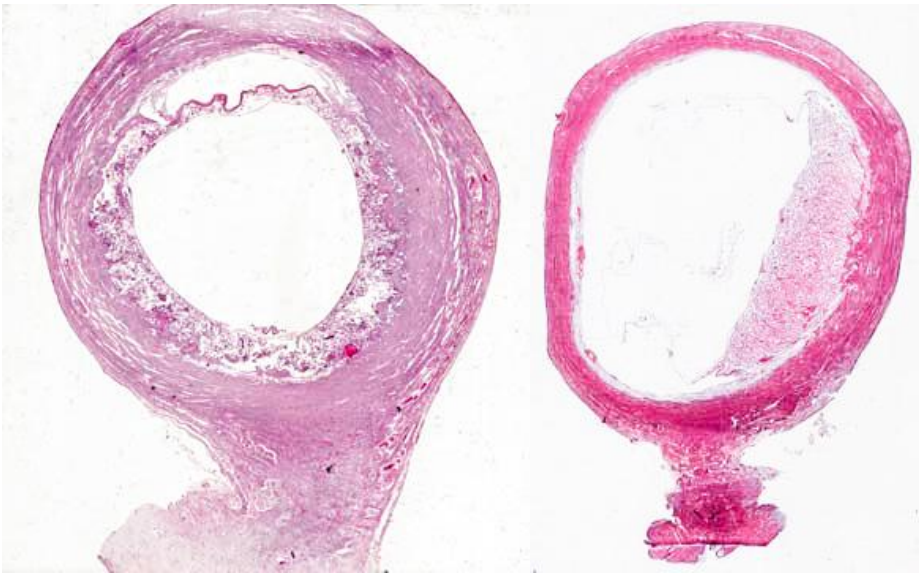


Figure 1.1: Boyd collection. Human embryo CR length 20mm and 90mm, 8.5 and 15.5 completed gestational weeks (low power) (7, 8).

In summary, placental development begins with the growth of trabecular columns formed by primitive syncytium, partially surrounding lacunar spaces. These columns become radially arranged and are subsequently invaded by cytotrophoblast. Secondary villi then develop. Spiral arteries within the myometrium do not open directly into the intervillous space: maternal blood flow (once established) is via gaps within the trophoblastic shell and drainage is via randomly distributed venous openings. With growth and development,

maternal arteries communicate with the intervillous space via random perforations of the basal plate. These arteries are invaded by cytotrophoblast. Chorionic villi, as they grow, have an invasive phenotype and can be seen lying within uterine veins. The placenta continues to grow in circumference throughout the pregnancy, at least in part by ongoing growth at the placental edge.

Growth of the placenta in the first trimester is supported by secretions from the endometrial glands delivered into the placenta through the developing basal plate and shows little variation within the human population (9). This form of nutrition, described as histiotrophic, is not dependent on maternal arterial circulation to the placenta: during the first trimester, maternal blood flow is prevented from entering the placental intervillous space by intravascular trophoblast occluding the tips of spiral arteries. Oxygen concentrations within the developing chorionic sac are thus relatively low. The first trimester is the period within which embryonic organogenesis occurs and the developing organs within this environment of relatively low oxygen concentrations are believed to be protected from teratogenesis mediated by reactive oxygen species (ROS) (10). In order to meet the energy demands required for growth and development of the metabolically active human fetus, the placental circulation derives from the maternal arterial circulation from the end of the first trimester. The subsequent threefold rise in intraplacental oxygen concentration permits high energy electron transfers, particularly within mitochondria, but also results in the increased production of potentially damaging ROS (11). The change in perfusion comes about through cytotrophoblast invasion of the decidual maternal spiral arteries, with replacement of the smooth muscle by fibrinoid material and increased maternal vessel diameter (12). Further placental growth through the second and third trimesters is via circumferential growth, with expansion of the villous tree as primary villi give rise to intermediate and terminal villi (13).

1.3.2 Normal histology

Standard light microscopic examination of the term placenta demonstrates the characteristic morphology of umbilical cord, membranes, chorionic plate, chorionic villi, intervillous space and decidua with included maternal vessels. The normal morphology of these structures has been widely reviewed in standard textbooks (3, 14).

1.4. MACROSCOPIC APPEARANCES OF TERM PLACENTA

A comprehensive review of all possible placental macroscopic findings is beyond the scope of this study. Standard textbooks (3, 14) were referred to throughout this study. The most relevant macroscopic placental findings are reviewed below.

1.4.1 Normal macroscopic placental findings

Placental disc

The normal placenta at term has a round to ovoid shape, with average weight 470g, average diameter 22cm and average thickness 2.5cm (14). The degree of variation in placental shape (placental eccentricity) is reported to be high (15, 16). The fetal surface is smooth, with vessels radiating from the point of umbilical cord insertion. The maternal surface represents the plane of separation of the placenta from the uterine wall - which occurs physiologically at the conclusion of the second stage of labour. The basal plate – the deepest part of the delivered placenta, is thus composed of a fusion of the deepest chorionic villi and the most superficial maternal decidua. The placental bed remains in utero, with admixed residual invasive trophoblast. The maternal surface of the placenta appears grooved: this appearance is attributable to folds of the basal plate extending upwards within the placental parenchyma. These folds are termed placental septa. The intervening aggregates of chorionic villi have a lobular form and are termed cotyledons. The macroscopic appearances of a serially sliced, normal term placenta are shown in Figure 1.2.

Figure 1.2. Serially sliced placenta, 39 weeks gestation, unremarkable cut surface.

Umbilical cord

The umbilical cord has a spiral or coiled structure, parallel to the course of the vessels within. Many studies identify one twist (complete turn through 360°) per 5cm length of cord as approximately the median rate of spiralling – the coiling index, more formally defined as the number of complete coils divided by the length of the cord in cm (17). The macroscopic appearances of normal coiling, hyper-coiling (>90th centile) and hypo-coiling (<10th centile) of the umbilical cord are illustrated in Figures 1.3 – 1.5. Approximately 90% of cords have a predominantly left-handed spiral or twist, with most of the remainder predominantly right-handed. A small proportion of cords lack any evidence of coiling at all - so-called “flat cords” (18).

Figure 1.3. Normally coiled umbilical cord, 39 weeks gestation.

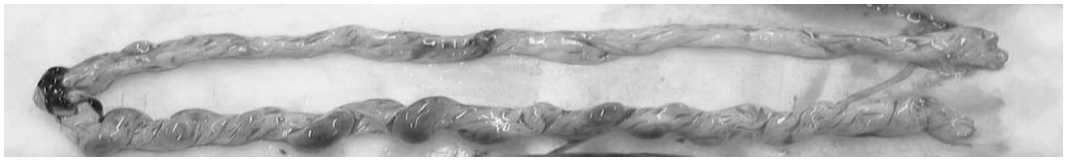


Figure 1.4. Hyper-coiled umbilical cord, 38 weeks gestation.

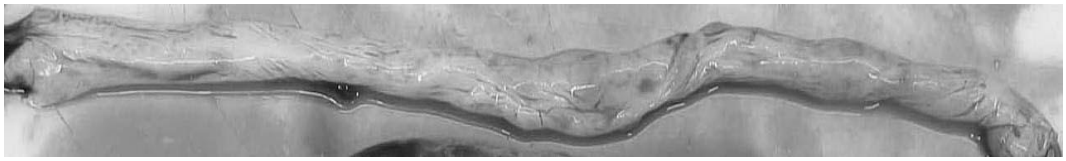


Figure 1.5. Hypo-coiled umbilical cord, 39 weeks gestation.

1.4.2 Macroscopic appearances of placental infarcts

Placental infarcts are variable in size, shape and distribution, but most commonly are seen as pale, roughly wedge-shaped lesions with the base lying just above the basal plate. Size ranges from a few millimetres to a centimetre or more, and location is most often peripheral, but may be located anywhere within the parenchyma (3). Recognition of infarcts relies on their increased density to palpation as well as their macroscopic appearance, which ranges from a dense dark red fresh lesion progressively to brown/yellow/white (3); although the appearances are quite characteristic, some commentators note the possibility of overlap of macroscopic features between infarcts and intervillous thrombi (qv) (19). The typical macroscopic appearances of placental infarction are illustrated in Figure 1.6. The incidence of macroscopic infarction reported in the literature is variable, but has been stated to fall into a range of 10-25% of delivered placentas (20). This has been questioned in a smaller study, which found a rate of 3% (4 /126 cases) in uncomplicated pregnancies at term (21).

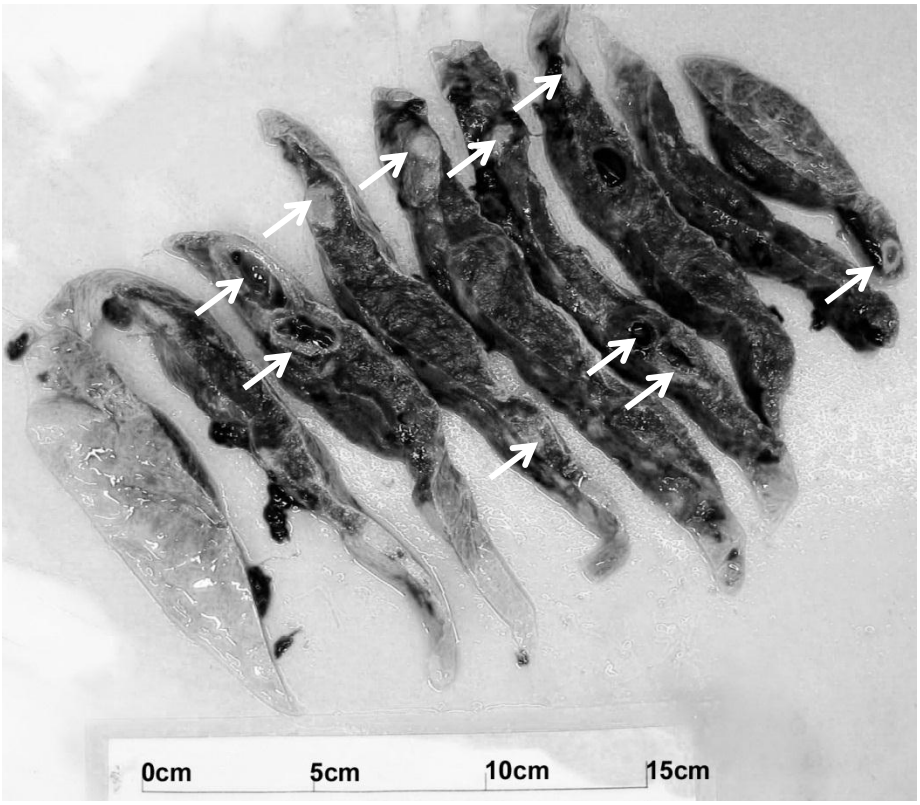


Figure 1.6. Serially sliced placenta, 39 weeks gestation, showing infarcts of varying age (arrows).

1.4.3 Macroscopic appearances of intervillous thrombi

Intervillous thrombi form a subset of all placental thrombi/haematomas, but are the most common thrombotic lesion, with an incidence of 14% in one study (19). Their macroscopic appearances are those of laminated thrombi, often multiple and distributed throughout the placental parenchyma (3).

1.4.4 Macroscopic appearances of fetal thrombotic vasculopathy

These lesions are characterized by a wedge of pale chorionic villi (22, 23), usually with the base of the wedge abutting the basal plate. It is said that the lesions are difficult to identify in the unfixed placenta, being much more apparent as an area of pallor following fixation (3).

1.5. MACROSCOPIC AND MICROSCOPIC FINDINGS IN TERM PLACENTA; CURRENT EVIDENCE FOR CORRELATION WITH ADVERSE CLINICAL OUTCOME

1.5.1 Variations in placental shape and cord insertion centrality

The clinical significance of placental shape variation is controversial. Increasing the deviation from true circularity has been reported in one prospective cohort to be associated with decreased placental efficiency (16), but similar assessment of shape variation was not found to be associated with a range of common obstetric outcomes (24). Variations in cord insertion site centrality are also of uncertain clinical significance, with suggestions of reduced placental insufficiency (24, 25) balanced against no demonstrable association with common obstetric outcomes (24). Velamentous cord insertion has also been associated with placental insufficiency (26).

1.5.2 Placental infarction

Placental infarction is recurrently reported to be associated with fetal growth restriction, both early and late onset. The majority of studies report outcomes in pre-term infants (27-32). Placental infarction has also been suggested as a possible risk factor for cerebral palsy in one study (33). Placental infarction at term is less commonly reported, but is also reported to be associated with fetal growth restriction (34) and some adverse maternal obstetric characteristics (20).

1.5.3 Chorioamnionitis and funisitis

Chorioamnionitis is defined as acute inflammation (polymorph mediated) of the placental membranes, while funisitis is infiltration of the umbilical cord tissues by a polymorph population. The histological diagnosis relies on routine haematoxylin and eosin staining of paraffin sections of membranes and cord, establishing the presence or absence of an acute inflammatory cell (predominantly neutrophil leucocyte) infiltrate in these tissues.

Migration of acute inflammatory cells in chorioamnionitis and funisitis follows a sequential pattern. The extra-placental membranes are first involved, followed by infiltration of the subchorionic fibrin, ie just below the chorionic plate. There is then extension of the infiltrate from subchorionic fibrin into the chorionic plate. The fetal

vessels within the chorionic plate subsequently become infiltrated by polymorphs and, lastly, the umbilical cord becomes involved: acute inflammatory cells infiltrate umbilical vessel walls and then extend into Wharton's jelly.

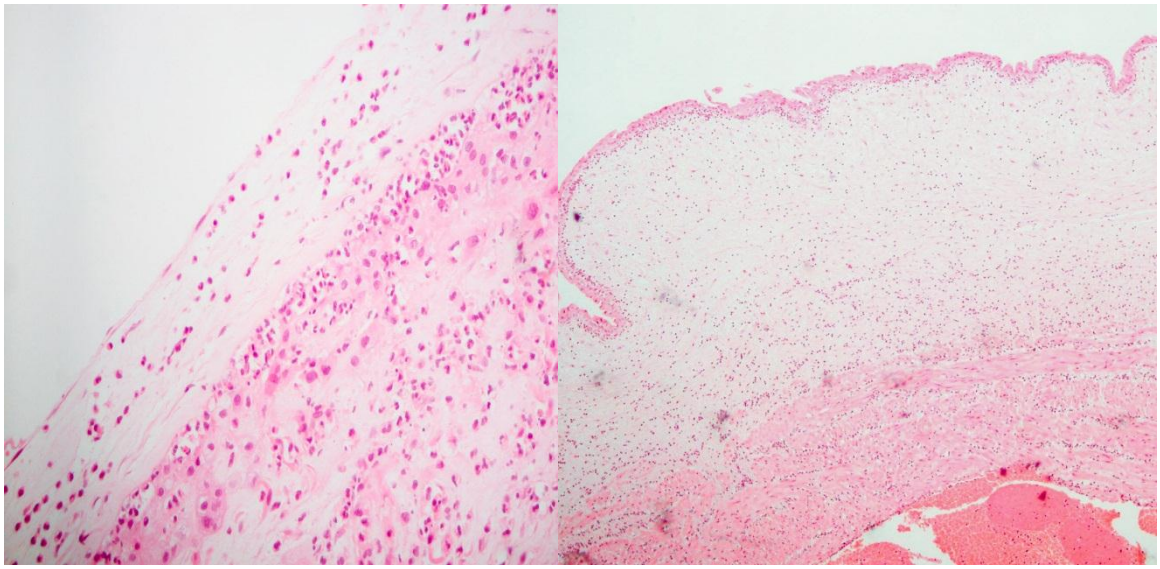


Figure 1.7. Photomicrograph of placental membranes (left) and umbilical cord (right) showing infiltrate of polymorphonuclear leukocytes, 40 weeks gestation (x 10 objective).

A number of studies, relying on the interpretation of fluorescence in situ hybridization studies of the X and Y chromosomes in male infants, have been carried out, to determine the origin of the inflammatory response in each micro-anatomical compartment. These demonstrate that maternal polymorphonuclear leukocytes form the predominant group of inflammatory cells in the extra-placental membranes and subchorionic fibrin, while fetal polymorphonuclear leukocytes form the predominant group in the infiltrate within the umbilical cord and chorionic plate (35). This demonstration may, in practical terms, be relied on to support differentiation of maternal from fetal inflammatory response in H&E-stained sections. Figure 1.7 shows the histological features of inflamed membranes (maternal inflammatory response) and inflamed umbilical cord (fetal inflammatory response).

Histological assessment of membranes and cord has been subject to a number of grading systems, mostly in research settings. The most commonly cited system (36) of identifying various reaction patterns of inflammation describes both maternal and fetal inflammatory response in terms of stage and grade, with stage corresponding to degree of progression of inflammation and grade relating to the intensity of the infiltrate. Stage is subdivided into three groups (early, intermediate and advanced) and grade into two (mild-moderate and severe). It should be noted that, for the twenty cases which formed the basis of this

classification, overall interobserver agreement stood at 81%. In addition, while there is some evidence supporting the association of severe grade inflammation with clinical outcome, the overall clinical significance of this classification system remains untested on a normal population

The true incidences of histological chorioamnionitis and funisitis vary according to the population characteristics. Histological chorioamnionitis appears to have a definite relationship with gestational age, the highest frequency affecting spontaneous miscarriages at 20–24weeks of gestation (around 80–90% of cases), falling to around 10% of deliveries at term. The integrity of the placental membranes is also important: histological chorioamnionitis is identified in around 30% of all preterm labour with intact membranes but rises to 50% of those presenting with preterm premature rupture of membranes (37, 38). Longer duration of labour is also associated with increased frequency of histological chorioamnionitis, being reported in 20–30% of deliveries following labour compared to <5% delivering at term with no labour (39, 40).

1.5.4. Histological changes reported as markers of impaired perfusion

Terminal chorionic villi may appear both small for the gestational age and widely spaced (with increased intervillous space): this histological lesion is said to be associated with impaired maternal perfusion (41). The lesion is also known as accelerated villous maturation or distal villous hypoplasia, descriptive terms encompassing what is hypothesized to be the adaptive nature of the histological appearance in the face of reduced perfusion.

Maternal vasculopathy is defined as persistence of muscularisation (i.e. absence of physiological conversion by means of trophoblast invasion) at term, and also atherosis characterised by fibrin and necrosis of the arterial wall together with an intramural accumulation of lipid-laden macrophages and perivascular lymphocytes. These changes are relatively rarely seen in the intra-decidual segment of the placental bed spiral arteries, being more detectable in arteries deeper within the maternal arterial bed (3).

Chorangiosis is defined histologically as multiple (at least 3) areas within the placenta where 10 chorionic villi contain 10 or more vessels, in non-infarcted zones. The incidence in an unselected population was reported to be 5.5% (42).

Syncytial knots are morphologically recognizable as dense aggregated nuclear material within syncytiotrophoblast, at the periphery of chorionic villi. Historically, these have

been regarded as evidence for uteroplacental ischaemia or fetal stress, although more recent discussions have centred around their role in placental apoptosis (43).

A placental infarct is a localised area of ischaemic villous necrosis. They may be central, peripheral or both: most are roughly triangular, with the base adjoining the basal plate. Ovoid or spherical infarcts are less common. Infarcts may be of varying age. The macroscopic and microscopic appearances are variable. Fresh infarcts are macroscopically red, becoming white as they mature. Histologically, an early infarct shows aggregated villi with obliterated intervillous space (illustrated in Figure 1.8), maturing into an old infarct, composed of crowded avascular villi (3). The incidence of placental infarcts is reported to be between 10 and 25% in uncomplicated pregnancy (19, 20), although may be less in the setting of more modern obstetric care (21). The instance is much higher in hypertension, varying between 34% in cases of mild pre-eclampsia to nearly 70% in severe hypertension. Central infarcts also appear to be more associated with significant hypertension (3, 44).

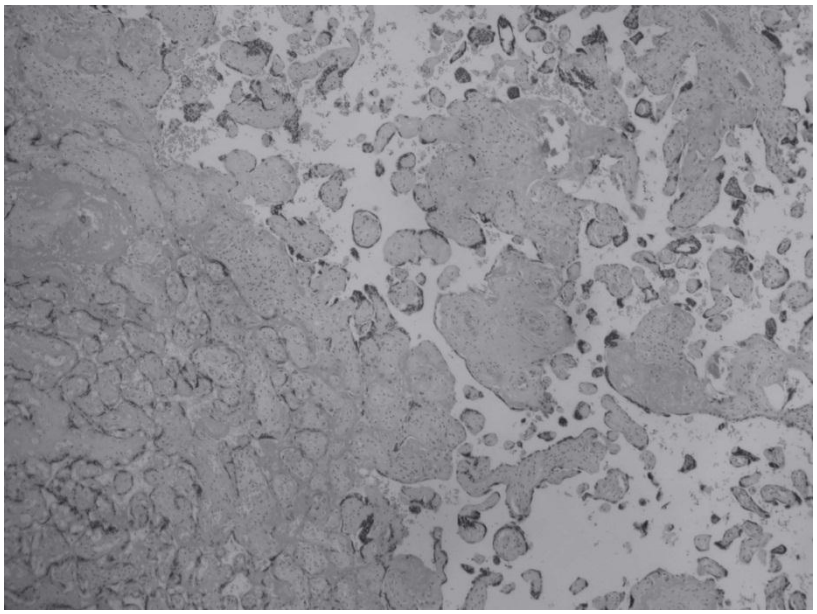


Figure 1.8. Photomicrograph of aggregated villi surrounded by fibrin (left) in placental infarction, 38 weeks gestation (x 4 objective).

1.5.5 Villitis of unknown etiology

Villitis of unknown etiology (VUE) is a descriptive term employed to describe chronic inflammatory lesions within villi for which the underlying cause is unknown (45). The lesions are most often noted in placentas of 32 weeks gestation or later (46). The infiltrate has been reported to be composed of CD 68 positive macrophages and a T-cell population of maternal origin (47). Plasma cells are not a feature. There is believed to be an

association between these lesions and intrauterine fetal growth restriction (48) and possibly also recurrent reproductive loss (46), although the lesion in these recurrent cases is more often now recognized as intervillous histiocytosis (49).

Histological classification of the lesions of VUE is most often based on the number of chorionic villi involved and their location within the placental structure. One grading system current within the literature separates the process into low-grade and high-grade lesions, depending on the location within the placental disc on histological examination and the number of chorionic villi involved (46).

The incidence of VUE is often quoted as between 5% to 15% of placentas (50).

1.5.6 Fetal thrombotic vasculopathy

This lesion is defined as thrombus formation within a chorionic plate or fetal stem artery with downstream avascular chorionic villi (51). These histological lesions are illustrated in Figure 1.9. While these lesions may be macroscopically visible, the current literature favours reliance on the microscopic appearances of the placenta (23).

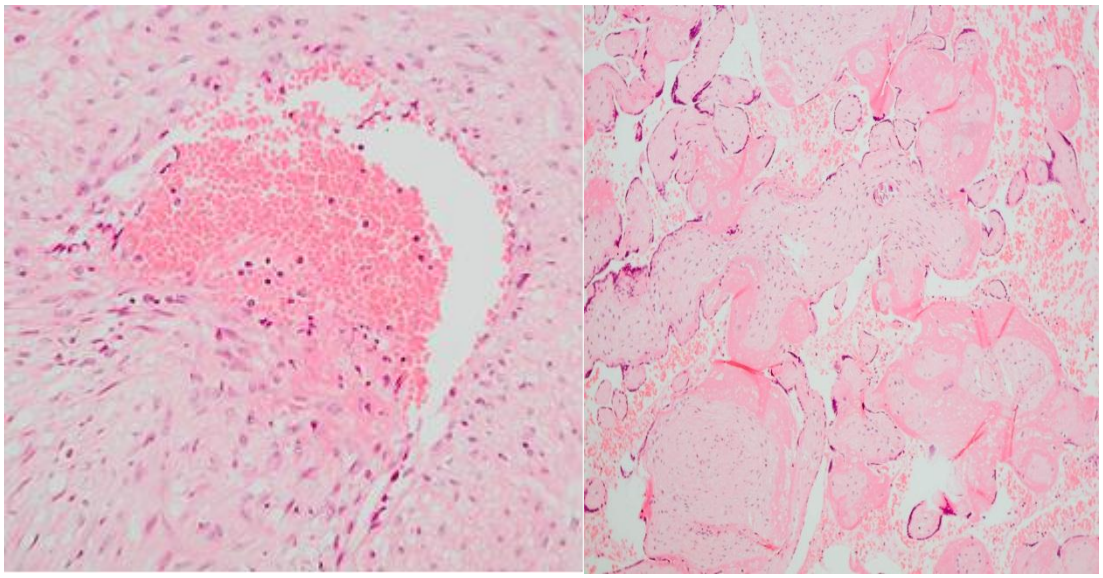


Figure 1.9. Photomicrograph of chorionic plate vessel with luminal fibrin adherent to endothelium (left) and downstream avascular chorionic villi (right), 38 weeks gestation (x 10 objective).

The incidence of the lesion is uncertain, given that some studies include placentas from stillbirths, where the vessels show changes which may be better regarded as post-mortem

involutionary change. Where stillbirths are excluded, the incidence of this lesion has been reported as between 3 and 10/1000 (22).

1.5.7 Histological markers of placental abruption

Placental abruption is a clinical event where the placenta separates prematurely from the uterine placental bed; it is associated with a range of adverse perinatal outcomes (52). Unifying clinical diagnostic criteria are, however, perceived to be absent from the clinical literature, although recently proposed inclusion criteria for a clinical diagnosis of abruption include sonographic visualization of abruption, evidence of retroplacental clot and vaginal bleeding accompanied by non-reassuring fetal status or uterine hypertonicity.

The aetiology of abruption is not well understood but there are some reports linking abnormal trophoblast invasion of spiral arteries with spiral artery rupture and thus premature separation of the placenta.

Where correlation between clinical findings and pathological observations has been attempted, diagnostic concordance has been reported to be poor. The lesion most often cited as a pathological finding in abruption is the presence of a retroplacental blood clot on macroscopic placental examination: this has been reported in 77% of clinically diagnosed cases in one study (53).

Candidate histological inclusion criteria suggested for a positive diagnosis of abruption include haematoma, fibrin deposition, compressed villi and (in older cases) haemosiderin-laden macrophages. These criteria were found to be only 30% sensitive although 100% specific. Of other histological lesions raised as possible associates of clinical placental abruption, placental infarction has been identified as reaching statistical significance (49). Chorioamnionitis has been associated with clinical abruption, but not with the identified histological markers of abruption (53).

1.5.8 Chronic histiocytic intervillous inflammation

Chronic histiocytic intervillositis (CHI) is a relatively recently described placental abnormality, first reported in 1987 (54, 55). It is rarely reported within the literature, with 69 affected pregnancies identified in a recent systematic review examining this lesion with respect to recurrence risk (56). The variability within the literature relating to this lesion is perhaps emphasized by the literature search findings of the systematic review: 122 publications on the initial search were reduced to 6 suitable for inclusion in a systematic review. It is of clinical interest in that the lesion is thought to be associated with intrauterine growth restriction and fetal death, and may recur in subsequent pregnancies.

The histological appearances are characterised by an infiltrate within the intervillous space, composed of lymphocytes, histiocytes and monocytes, of maternal origin. When characterized immunohistochemically, the infiltrate is reported to be >95% histiocytic (CD68 positive), with <5% CD15 positive eosinophil granulocytes and CD3 positive T cell lymphocytes and <1% CD20 positive B cells (49, 57).

The incidence is uncertain; a rate of approximately 40/1000 has been reported for first trimester miscarriages with a normal karyotype and between 0.1/1000 and 0.6/1000 for second and third trimester placentas. The recurrence risk has been reported as 80% in subsequent pregnancies (49).

1.5.9 Massive perivillous fibrin deposition/maternal floor infarction

This lesion is characterised by the accumulation of a combination of polymerised fibrin and trophoblast-derived extracellular matrix products around large portions of the distal villous tree. This matrix is infiltrated by extravillous trophoblast, believed to be derived from a villous trophoblast. The process is analogous to the development of the anchoring villi at the base of the placenta, but when pathological is defined as between 30 and 40% of the placental parenchyma being obliterated by perivillous fibrin or (in the case of maternal floor infarction), the villi of the entire maternal floor are embedded in fibrin which is at least 3 mm thick (58, 59).

1.5.10 Intervillous thrombus

This lesion is defined histologically as laminated thrombus lying within the intervillous space. Single or multiple lesions may be present, each varying in size from 0.1cm to 5cm. The incidence of these lesions in an unselected population has recently reported to be 19% (19).

1.5.11 Plasma cell deciduitis

Plasma cell deciduitis – infiltration of decidualised endometrium by plasma cells – is a lesion of uncertain incidence and significance. Infiltration of endometrium by plasma cells in the non-pregnant uterus is associated with impaired maternal fertility. In pregnancy, the rate of plasma cell deciduitis has been reported to be increased in idiopathic preterm labour compared with controls (60), although recognition of the lesion amongst pathologists is variable (61).

1.5.12 Eosinophilic inflammation

This relatively recently recognized lesion, first described in 2002 (62), involves usually a single chorionic vessel surrounded and focally infiltrated by fetally derived eosinophils and CD3 positive T cells. A recent large series reports a possible association with villitis of unknown etiology (VUE, qv), but no association with adverse fetal outcome (63).

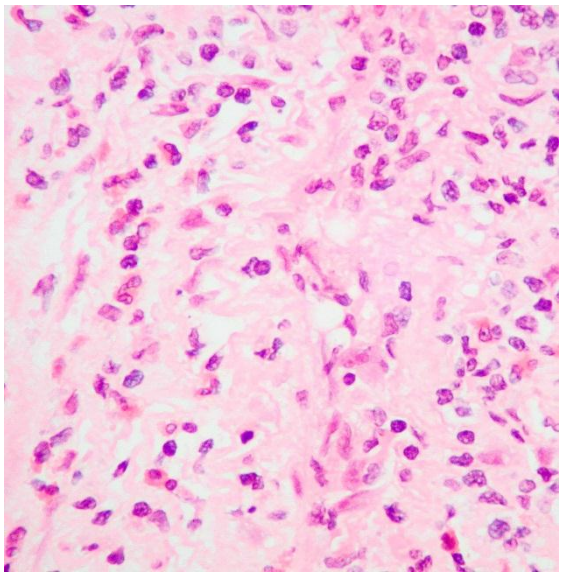


Figure 1.10. Photomicrograph of chorionic plate vessel wall showing infiltrate of small lymphocytes and eosinophils (x 40 objective).

1.5.13 Subacute chorionic plate inflammation

Subacute chorioamnionitis is a lesion which has been described within the literature in a small number of publications. The infiltrate is variously described as a subamniotic mononuclear cell infiltrate with occasional polymorphs (36), or a more severe lesion with mixed degenerative neutrophils and mononuclear cells (64). In the latter study, a positive association with chronic lung disease was reported.

1.6 PLACENTAL PATHOLOGY: MECHANISMS OF DISEASE

1.6.1. Immune responses

Neutrophil recruitment in acute inflammation is a multistep process (65), with neutrophils becoming initially loosely attached to “activated” endothelium in the locality of a stimulus via endothelial expression of selectins (66, 67), with subsequent tighter endothelial attachment mediated via leukocyte integrins (68). Both selectins and integrins are adhesion

molecule families, binding to complementary ligands when converted from low affinity to high affinity molecules in an appropriate cytokine environment.

Identification of specific components of the immune response is often by description of specific cell-surface glycoproteins and glycolipids – including, for example, the integrin and selectin families identified above. Antibodies raised against cell differentiation or clusters of differentiation (CD) molecules form the basis of immune cell classification (69). The immune response involves leukocyte-leukocyte interaction, leukocyte-nonimmune cell interaction, leucocyte-tissue matrix interaction and leucocyte-foreign antigen interaction, via this large collection of cell surface markers, now numbering >300. Specific CD subsets identify many specific immune cells: B (70) and T (71) cells are identified both by common markers (CD20 and CD 79a for B cells, CD 3 for T cells (69) and specific markers identifying B and T cell subsets – for example, T regulatory cells (72, 73).

Acceptance that the histological changes of chorioamnionitis and funisitis are attributable to infection is virtually universal, with a large number of studies linking this histological finding to ascending maternal genital tract infection/colonization (74-76). Previous candidate factors such as fetal hypoxia, changes in the pH of the amniotic fluid or of the irritant effects of meconium have been rejected on the basis of experimental study.

The cellular components of the inflammatory response in villitis of unknown etiology (VUE) have been demonstrated to be cells of monocyte/macrophage lineage admixed with T cells (77). The chronic inflammatory response underlying this observation is also induced by expression of various cytokines (eg interleukin 1, interferon γ and tumour necrosis factor α) and cell surface adhesion molecules, interacting to induce lymphocyte and monocyte migration. It should be noted that the underlying event inducing cell migration in non-infectious villitis is unknown.

The complement system forms part of the humoral immune system. It is conventionally described in terms of classical (antibody-activated) and alternative (antigen-activated) pathways, with a third, lectin-activated, pathway also existing. All complement pathways are centred around activation of C3. The classical pathway is activated by antibody-antigen interaction and immune complex deposition. Carbohydrate ligands (including those found on some micro-organisms) and injured tissue undergoing apoptosis and necrosis activate

the lectin pathway. The alternative pathway is activated by cell surface components of micro-organisms and by IgG. The pathways are highly complex and involve more than 30 plasma proteins, interacting with a range of cell surface receptors (78-80). Apparent activation of complement systems has been reported in pre-eclampsia (81, 82).

1.6.2 Impaired perfusion

As has been noted above, the normal term placental decidua is characterized histologically by the presence of transformed maternal vessels. This process begins in the second half of the first trimester, with migration of cytotrophoblast from the basal aspect of anchoring villi, invading decidua and myometrium (extravillous cytotrophoblast) and forming intra-luminal cellular plugs within decidual spiral arteries (intravascular extravillous cytotrophoblast). The endovascular cytotrophoblast subsequently invades and destroys the maternal endothelium and arterial smooth muscle: the endpoint of this process is the transformation of small muscular arteries to dilated, thin walled vessels containing fibrinoid within their walls (83).

Failure of this physiological process, with deficient intramural trophoblast invasion and hyperplastic (or non-transformed) muscular arteries, has been recognized to be a hallmark of pre-eclampsia (84). The initial pathological event is considered most likely to be an abnormal immunological interaction between fetal trophoblast and maternal natural killer (NK) cells (85). Maternal blood flow into the intervillous space is consequently markedly compromised: decreased placental perfusion is thought to interact with maternal constitutional factors to result in oxidative stress, endothelial activation and multisystem maternal disease (86).

1.6.3 Oxidative stress

Oxidative stress is a process universal amongst all aerobically respiring organisms. The high-energy electron transfers taking place within cellular organelles support oxidative phosphorylation but also generate reactive oxygen species which are toxic to biological molecules within the cell. The potential pathogenicity of reactive oxygen species was first demonstrated in 1952, when it was shown that increases in oxygen pressure could cause chromosomal aberrations in radiation-exposed pollen grains (87). Generation of reactive oxygen species (ROS) induces DNA, protein and lipid damage if the homeostatic equilibrium between pro-oxidant and antioxidant events becomes unbalanced. Oxidative

damage to biomolecules has since been implicated in a wide variety of pathophysiological processes in humans, including ageing, neoplasia, degenerative diseases, autoimmune diseases and neurological dysfunction (88, 89). The interaction between oxidative stress generated within mitochondria and intracellular stress occurring within separate organelles such as the endoplasmic reticulum is also likely to be important in pathological processes within the placenta (90).

There is an equilibrium within the normally functioning cell between the generation of reactive oxygen species and antioxidant defences: loss of balance in this homeostatic process may induce cell damage and ultimately cell death. The most common reactive oxygen species is considered to be the superoxide anion, resulting from leakage of electrons onto molecular oxygen from the respiratory chain within mitochondria. There is a shorter electron transport chain within in the endoplasmic reticulum; the formation of disulphide bonds during protein folding is an oxidative process and also generates superoxide anions. Under conditions of endoplasmic reticulum stress, repeated attempts to refold misfolded proteins may take place. It is recognised that intracellular stress - such as hyperoxia, hypoxia or hypoglycaemia - may result in oxidative and endoplasmic reticulum stress, with possible subsequent induction of apoptosis pathways (91).

Understanding of the cell cycle and the pathways involved in endoplasmic reticulum homeostasis is useful in evaluating such pathological processes. A biologically stressed tissue will exhibit reduced cellular proliferation and increased cell death, and immunohistochemical markers of these processes will identify these processes within tissue sections. Amongst the immunohistochemical markers available to characterize these processes is cleaved caspase 3.

Caspase 3 is a target protein, subject to irreversible proteolytic cleavage, in a complex network of downstream signalling pathways, triggered by engagement of cell surface receptors (92). This is in contrast to conventional signal transduction is mediated by ion fluxes, secondary metabolites, protein-protein interactions and post-translational protein modifications: these are often subject to phosphorolytic and ubitquinolytic regulation and as such are reversible (93). The caspase family (12 caspases have been described to date) is best known for its function in apoptosis signalling, although there may also be involvement in some inflammatory gene regulation (94).

1.7 CURRENT DEFINITIONS OF CLINICAL PRESENTATIONS: CLINICAL DEFINITION AND CLINICAL INCIDENCE

As will be further discussed in section 8, clinical definitions of specific obstetric, fetal and neonatal disorders can be variable. In this review, definitions and clinical practice current within the United Kingdom form the basis for discussion.

1.7.1 Maternal/fetal chorioamnionitis

The clinical criteria for the diagnosis of chorioamionitis include maternal pyrexia (above 37.8°C), maternal tachycardia, leukocytosis (sensitivity 29-47%, false positive rate 5-18%), uterine tenderness, offensive vaginal discharge and fetal tachycardia (a rate above 160 beats/minute) (95). The incidence of clinical chorioamnionitis is reported to be between 0.5%-10% of all pregnancies: 1-7% at term and 10-20% of preterm (<33 weeks) deliveries (96-98).

1.7.2 Pregnancy-induced hypertension/pre-eclampsia

Hypertensive disorders during pregnancy occur in women with pre-existing primary/secondary hypertension, and in women who develop new onset (pregnancy-induced) hypertension ie blood pressure ≥140/90mmHg after 20 weeks gestatation.
Pre-eclampsia is defined as pregnancy-induced hypertension with diastolic blood pressure ≥ 90mmHg on at least 2 consecutive occasions, 4 hours apart, or a single episode of diastolic blood pressure ≥ 110mmHg on one occasion, the hypertensive events to be associated with proteinuria (greater than 0.3 g/24 hours).
Severe pre-eclampsia and eclampsia (defined as the occurrence of 1 or more convulsions superimposed on pre-eclampsia) are relatively rare, but serious, complications of pregnancy, with approximately 0.5% of pregnancies associated with severe pre-eclampsia and 0.05% with eclampsia. Eclampsia has a maternal mortality rate of approximately 1.8%, with a further 35% of mothers experiencing a major complication (99-101).

1.7.3 Placental abruption

Placental abruption is a clinical event where the placenta separates prematurely from the uterine placental bed; it is associated with a range of adverse perinatal outcomes. Unifying clinical diagnostic criteria are, however, perceived to be absent from the clinical literature,

although proposed inclusion criteria for a clinical diagnosis of abruption include sonographic visualization of abruption, evidence of retroplacental clot and vaginal bleeding accompanied by non-reassuring fetal status or uterine hypertonicity (102).

1.7.4 Fetal growth restriction

Fetal growth restriction is defined as fetal growth which has failed to achieve the true growth potential in that pregnancy. A broader group of fetuses, those small for gestational age, includes both growth restricted fetuses and those fetuses which are constitutionally small. Both growth restricted and small for gestational age fetuses are at greater risk of stillbirth, birth hypoxia, neonatal complications, impaired neurodevelopment and possibly late complications in adult life (including type 2 diabetes and hypertension).

The clinical definition is based on biometry, with the most commonly used threshold that of the 10 percentile for abdominal circumference and estimated birth weight on antenatal ultrasound scan.

1.8 EVIDENCE FOR CORRELATION BETWEEN CLINICAL DISEASE AND HISTOLOGICAL CHANGES

Evaluation of the current evidence correlating clinical obstetric or neonatal problems with placental morphological change is highly problematic. Diagnostic criteria for specific clinical problems are very variable within the literature and occasionally clinical problems correlated to specific placental findings are referred to descriptively but not defined. There is a proliferation of non-standardised terminology and disease entities are often referred to without a clear statement as to whether it is the clinical or histological process under discussion.

1.8.1 Maternal/fetal chorioamnionitis

Preterm labour accounts for around 10% of all deliveries, having multiple well-recognised clinical and epidemiological associations, amongst which chorioamnionitis is clearly established (103-106). A small number of studies have examined the question of recurrent preterm birth and placental inflammation (107, 108). These studies are more difficult to place in context: the histological scoring systems used for the studies are variable, and fetal and maternal inflammatory responses have not always been reported separately.

Overwhelming maternal sepsis is occasionally but consistently reported as a complication of chorioamnionitis (109). It is a relatively rare occurrence, forming only a small proportion of pregnancy-related intensive-care admissions; prognosis is generally good with appropriate treatment.

The associations between chorioamnionitis and stillbirths are controversial (110-113). In the specific group of preterm non-macerated stillborn infants with histological evidence of an established fetal inflammatory response (see below), it is reasonable to conclude that ascending infection was likely to be the underlying cause of the pregnancy loss, by precipitating delivery. This specific group accounts for only around 10-25% of all stillbirths (114), with the lower end of the range likely to be most applicable to Western populations (112).

The most important pathway relating chorioamnionitis to adverse neonatal outcome appears to be the fetal inflammatory response syndrome (115, 116). The data are, however, somewhat paradoxical. Histological chorioamnionitis is associated with improved survival in very preterm infants compared to gestationally age-matched cases with no inflammation (117), presumably as a consequence of the fact that preterm delivery is associated with ascending infections are otherwise normal infants, whereas those delivered preterm for other indications will very likely have other complications such as fetal growth restriction.

Assessing the implications of chorioamnionitis for all liveborn infants, born preterm or term, continues to be problematic. A meta-analysis carried out 10 years ago (118) reported significant associations between clinical chorioamnionitis and both cerebral palsy and periventricular leucomalacia in preterm infants and an association between histological chorioamnionitis and periventricular leucomalacia in preterm infants. For term infants, there was a positive association between clinical chorioamnionitis and cerebral palsy. A subsequent extended meta-analysis (119) reported an association between recorded clinical chorioamnionitis and cerebral palsy with a random effects model, and a more recent meta-analysis (120) reported associations between both clinical and histological chorioamnionitis and cerebral palsy in studies predominantly, but not exclusively, of preterm infants.

1.8.2 Pregnancy-induced hypertension/pre-eclampsia

Both clinical and experimental studies have reported links between clinical pregnancy-induced hypertension, pre-eclampsia and eclampsia and placental morphology. A retrospective case series has reported correlation between the clinical severity of the maternal hypertensive disorder and the degree of placental infarction present: infarction involving ≥5% of placental tissue was found in 39.7% of severe pre-eclampsia, 17.1% of mild pre-eclampsia and 5.1% of normal controls. When comparing placentas in severe pre-eclampsia, mild pre-eclampsia and normal mothers, there was an increase in the presence of any infarction and decidual arteriopathy (121).

A similar study, evaluating placentas from patients with severe pre-eclampsia with and without hemolysis, elevated liver enzymes, and low platelets (HELLP) syndrome has reported that infarction, intervillous thrombosis and abruption were more common in the pre-eclampsia group than in the HELLP group (122).

Analysis of placental microarchitecture of capillary loops in placentas from growth-restricted fetuses has shown a reduction in capillary loop number, with significantly increased length and fewer branches, compared to controls. It is suggested that these findings are consistent with an increase in fetoplacental vascular impedance at the capillary level and may account for impaired gas and nutrient exchanges in pre-eclampsia (95).

1.8.3 Placental abruption

Positive associations between chorioamnionitis and placental abruption in preterm and term births are reported within the literature (53, 55). The hypothesis is that localised inflammation processes disrupt normal placental attachment. It should, however, be acknowledged that many cases of abruption are associated with other, well validated, epidemiologically derived factors such as previous abruption, hypertension (as noted above), ethnicity, smoking and cocaine use (123-125).

1.8.4 Fetal growth restriction

Fetal growth restriction is most commonly defined as fetal growth < 10^{th} centile for gestational age (126) , but growth below the 3^{rd} centile has also been proposed as a more reliable indicator for associated perinatal morbidity (127). A number of authorities have

also noted that basing assessment of growth entirely on a population basis misclassifies a number of fetuses as either small or large for gestational age (128-130).

The most commonly reported specific placental lesions reported in association with fetal growth restriction include those lesions associated with maternal hypertension and also high-grade villitis of unknown etiology and chronic intervillous histiocytosis.

Early onset pre-eclampsia has been reported to be more strongly associated with specific placental lesions (infarction and decidual vasculopathy) than later onset pre-eclampsia (30, 131). Villitis of unknown etiology is repeatedly reported to be associated with growth restriction, albeit with lower grade lesions of more doubtful signficance (46, 132, 133). Chronic intervillous histiocytosis has been reported in to be associated with growth restriction (27), although as discussed elsewhere this lesion may be more associated with recurrent pregnancy loss (49, 134).

1.9 CONCLUSIONS

- Normal placental development is well understood. The end of the first trimester marks a shift from histiotrophic-fuelled growth and development to maternal perfusion-mediated growth and development.
- The normal macroscopic appearances of the placenta are defined in terms of size, shape, weight and cord insertion site.
- The normal microscopic appearances of the placenta are defined as micro-anatomical compartments: cord, free membranes, chorionic plate, intervillous space, chorionic villi and maternal decidua.
- Macroscopic abnormalities of the placenta include infarction, intervillous thrombus formation and fetal thrombotic vasculopathy.
- The significance of variations in placental shape (eccentricity) and centrality of cord insertion site is uncertain.
- Microscopic abnormalities of placenta include chorioamnionitis and funisitis, impaired perfusion, villitis of unknown etiology, fetal thrombotic vasculopathy, placental abruption, chronic histiocytic intervillous inflammation, massive perivillous fibrin deposition and maternal floor infarction, intervillous thrombus, plasma cell deciduitis, eosinophilic inflammation and subacute chorionic plate inflammation.
- Mechanisms of disease in placental disorders include acute and chronic inflammatory pathways, complement activation and impaired perfusion with subsequent oxidative stress.
- Associations between placental lesions and adverse clinical outcomes have been reported in the context of both prospective and retrospective studies. Findings are often confusing, conflicting and difficult to apply to clinical practice.

CHAPTER TWO: MATERIALS AND METHODS

Summary:

- Study design, recruitment of subjects and clinical data collection
- Macroscopic placental examination
- Computer based image analysis techniques
- Histological reporting protocols
- Immunohistochemical techniques
- Adaptation of systematic review construct
- Statistical analysis of data

2.1 STUDY DESIGN

The study design was single-centre, prospective, and of an unselected population, approved by Cambridgeshire Research Ethics Committee (Ref.no:07/Q0106/51, see Appendix A). All potential subjects were offered a patient information leaflet and written consent (Appendix B) was obtained from all participants within the study. Recruitment was carried out over a 13 month period from 2007 to 2008. Only subjects with an ongoing singleton pregnancy were eligible for inclusion in the study.

2.2 MATERIALS AND METHODS

2.2.1 Collection of placentas

At delivery, placentas delivered from participants consenting to inclusion in the study were inspected grossly by the delivering clinician/midwife, in keeping with pre-existing local clinical guidelines. Specific guidance relating to this study, to support the local midwifery staff, was provided in sluice areas. Briefly, placentas were placed in a labelled double bag within 20 minutes of delivery, which was kept in a dry, clean 1-2 litre plastic container with an airtight lid and in a fridge with the temperature maintained at 4-6°C. Daily transfers from the obstetric to the histopathology department were undertaken, together with a study-specific request form (Appendix C).

2.2.2 Collection of clinical data

Obstetric and neonatal outcomes for this project were collected onto a database specifically created for the study. The database was held on two password-protected servers (one for obstetrics and one for histopathology) within Addenbrooke's Hospital. A broad range of data for each mother and infant was collected, including demographics, pre-conception history, events in pregnancy, delivery and immediate neonatal outcome. The clinical parameters recorded for each mother and infant are summarized in Table 2.1

Collection of placentas and recording of clinical data were carried by Dr Sangeeta Pathak, clinical research fellow, Department of Obstetrics and Gynaecology, Addenbrooke's Hospital, who undertook a clinically based research project on this cohort.

Maternal characteristics	Infant characteristics
maternal age	sex
parity	gestational age
ethnic origin	birth weight
pre-existing essential hypertension	apgar scores at 1 and 5 minutes
smoking status	birth weight < 10th centile
pre-conception diabetes	suspected neonatal sepsis
hypertensive disorders of pregnancy	neonatal acidosis
suspected maternal chorioamnionitis	admission to neonatal intensive care
clinical abruption	
onset of labour	
mode of delivery	

Table 2.1 Clinical parameters recorded to assess maternal and infant outcomes

2.2.3 Protocol for macroscopic placental examination

Placental weight is usually routinely recorded as part of the macroscopic examination of the placenta, and is routinely recommended in guidelines issued in both the US and the UK (135, 136). It should be noted, however, that while fetal weight and placental weight tend to be correlated, placental weight alone may not be a useful predictor of function (137), with decreased placental/fetal weight ratios possibly reflecting the endpoint rather than the underlying cause of restricted growth of the fetal-placental unit in any individual pregnancy. There is equally limited evidence to support independent significance for placental measurements, which are closely correlated with placental weight (138). Macroscopic assessment of the normal and abnormal (infarcts, fibrin, intervillous thrombus etc) placental cut surface is described in the US and UK guidelines cited above.

For this study, the available publications describing placental examination were reviewed in the context of the study aims and objectives. A protocol was agreed within the research team (Appendix D).

Briefly, placentas were photographed, examined and blocked for microscopic examination by one of two researchers, assisted by a research assistant recording the information on a standardised proforma (Appendix 4). The cord was removed at the point of insertion into the placental disc. Blood was drained from the disc via the cord insertion point and any adherent clot removed from the maternal surface. A trimmed, drained weight was recorded,

together with measurements in 3 dimensions. The presence or absence of infarcts, intervillous thrombus, haematoma, macroscopically identifiable fibrin and maternal surface thrombus/blood clot was noted. The placenta was then sliced in parallel 1 cm intervals.

Photography was undertaken by recording pictures of the intact fetal and maternal surfaces, with the umbilical cord detached and included in the picture. A further photograph was taken following parallel 1 cm slicing, to record the appearances of the cut placental surface.

The cord was examined by recording length, diameter, coiling index (turns/length), direction of cord spiral, cord insertion site of the presence of absence of true cord knots.

The standard blocks sampled for histological examination included 3 of cord (maternal end, fetal end and central), membranes (including free edge), 3 full thickness central parenchymal blocks, 1 full thickness peripheral parenchymal block and 2 of the maternal surface. Any focal specific lesion was also sampled for histological examination.

Placental prosection was divided between the author and Dr Sangeeta Pathak, clinical research fellow, as part of the clinical research project noted above.

2.2.4 Eccentricity and cord centrality indices

Calculation of placental eccentricity and cord centrality indices was undertaken by Dr Sangeeta Pathak, clinical research fellow, again as part of the clinical research project noted earlier in this Chapter.

Briefly, photographs of the fetal surfaces of the placentas involved were analysed by computer based analysis. The widest possible X and Y axes, intersecting at right angles, were created. These are represented by A and B in the diagram below. The cord insertion point was mapped by two further X and Y axes, parallel to the originals, but intersecting at right angles at the cord insertion point (C and D in the diagram below). Mathematical formulae were created to calculate:

1. distance of umbilical cord insertion from the centre
2. cord centrality index (the smaller the index, the closer the cord insertion point to the centre)
3. eccentricity index, between 0 and 1 (a perfect circle has a value of 0; the more elliptical the shape, the closer to 1).

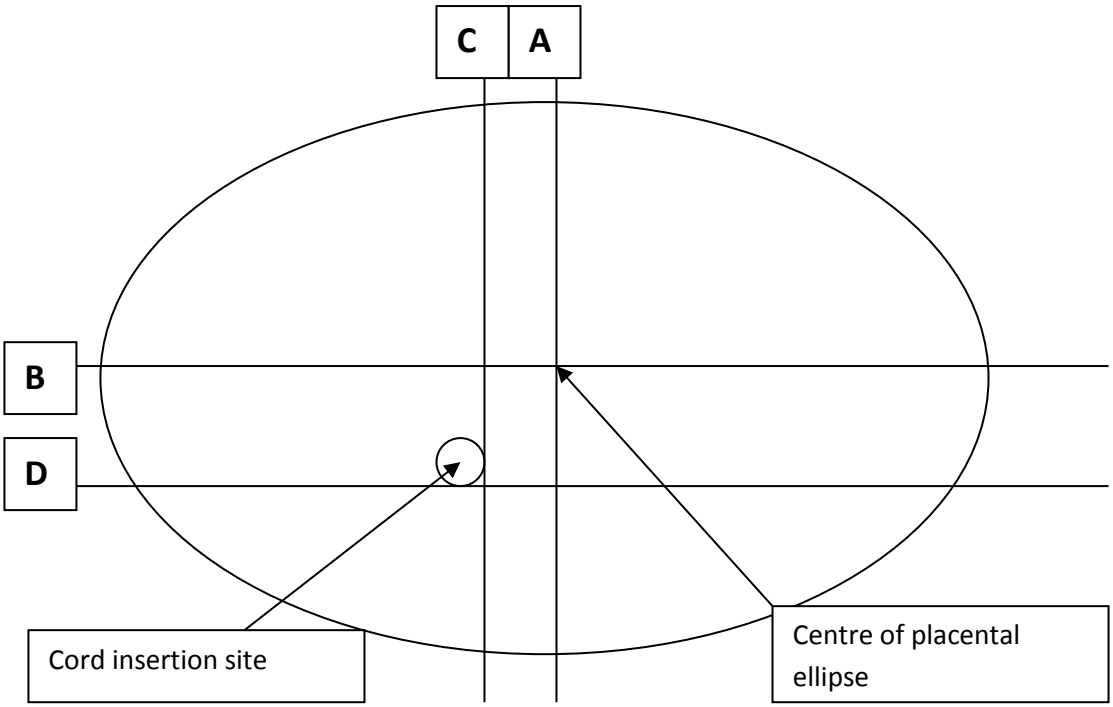


Figure 2.1. Diagrammatic representation of placental ellipse, showing axes intersecting at the centre of the ellipse (A and B) and at the cord insertion site (C and D).

2.2.5 Analysis of infarction images

The percentage of parenchymal infarction in the macroscopically identified infarct cases was analysed using ImageJ software, public domain Java based image analysis software developed by the National Institute of Health (139). The photographs of infarct cases were reviewed, with the total cut surface area and area of cut surface infarction determined as follows:

1. Set scale: analyze>set scale>measure known distance on scale included in photograph.
2. Known distance entered, calibrated with given number of pixels, scale set
3. Freehand tool selected, outline of each slice made: area measured: analyze>measure>area.
4. Repeat process for infarcts: freehand tool selected, outline of each infarct made: area measured: analyze>measure>area.

2.2.6 Protocol for processing tissue blocks

Tissue blocks were processed, sectioned and stained in accordance with standard laboratory protocols: following 24 hours fixation in 10% buffered formalin, standard tissue processing (Excelsior) and paraffin embedding, a full face tissue section was cut from each paraffin embedded tissue block. Standard haematoxylin and eosin staining was then undertaken. Following appropriate crosschecks between block, slide and study number, slides were issued to the reporting pathologist. Residual placental tissue was disposed of (following checks to confirm successful processing of tissue blocks) by incineration. The laboratory protocols adhered to are archived electronically as standard operating procedures (SOPs) within Addenbrooke's Hospital Histopathology Department and are subject to external review and audit by Clinical Pathology Accreditation UK (most recent CPA accreditation November 2011). Tissue block processing, cutting and staining were undertaken by members of the biomedical science team in the Department of Histopathology, Addenbrooke's Hospital.

2.2.7 Histology reporting protocols - initial analysis

Many previous studies of placental pathology have, as discussed in Chapter 1, been undermined by insufficiently rigorous definitions of histological abnormalities. A reporting schedule was thus agreed between the two lead reporting pathologists (Dr FA Jessop (FAJ), Addenbrooke's Hospital and Professor NJ Sebire (NJS), Great Ormond Street Hospital for Sick Children), reviewed and supported by Dr CE Hook, consultant paediatric/perinatal pathologist, Addenbrooke's Hospital. The objective of this first round of analysis was to place each case in one of eleven diagnostic categories which were found to be robustly reproducible, in line with current clinical reporting practice in the home institutions of both pathologists and supported by the current best evidence for the defining histological characteristics of each diagnostic group: this evidence has been discussed at length in Chapter 1 and the morphological features are summarized in Table 2.1. It should be noted that this phase of analysis thus excluded some placental histological changes (eg syncytial knots) where the current literature lacks sufficiently clear definitions and the reproducibility of the diagnostic criteria is doubtful (3).

Histological diagnosis	Morphological characteristics
no significant histological abnormality.	normal membranes, umbilical cord, maturation of chorionic villi within normal limits, normally transformed maternal vessels
features of ascending genital tract infection.	infiltration of neutrophil or eosinophil leucocytes into extra-placental membranes and/or chorionic plate and/or umbilical cord; apparent at low power microscopy
features of chronic placental underperfusion, including pre-eclampsia *	distal villous hypoplasia placental infarction maternal vasculopathy
villitis of unknown etiology	focal mononuclear cell infiltration of chorionic villi ± fibrinoid necrosis; apparent at low power microscopy
fetal thrombotic vasculopathy *	thrombi in stem villous arterial lumina ± mural fibrin in stem villous arteries ± downstream avascular villi
features of acute abruption *	retroplacental blood-clot
chronic histiocytic intervillositis	intervillous infiltrate of mononuclear cells not associated with villous infarction
massive perivillous fibrin deposition *	fibrin-entrapped, widely separated terminal/stem villi ; fibrin obliterates intervillous space
intervillous thrombus *	laminated thrombus within intervillous space
chorangiosis	≥ 10 capillary cross-sections involving ≥ 10 villi in ≥ 2 separate areas of the placenta
chorangioma *	discrete nodule of small thin walled blood vessels within loose perivascular stroma

Table 2.2: Histological characteristics of diagnostic groups

* Sampling protocols were drawn up to ensure that lesions which rely on correlation with the placental macroscopic findings were reliably and representatively sampled for histological assessment, please see Appendix 4.

A pilot cohort of 100 cases was reported, within the framework of this classification, independently by FAJ and NJS, supported by two rounds of face to face consensus

meetings. Interobserver agreement was reached in all 100 cases following this process. All study cases were then reported by one of the two researchers (including the 100 pilot cases, which were returned to the pool and randomly distributed between the two reporting pathologists).

2.2.8 Histology reporting protocols – sub-analysis

Sub-analysis of two histological groups - histological features of ascending infection and villitis of unknown etiology - was undertaken in this study.
This was preceded by histological review of all study cases initially classified as showing no significant histological abnormality. The principal objective of this rescreening of cases was to capture all cases showing degrees of inflammation which had fallen short of the original diagnostic criteria for a pathological diagnosis, and had thus been placed in the diagnostic category of "no significant histological abnormality" in the first round of analysis. Cases were then placed in additional subgroups as follows in Tables 2.3, 2.4 and 2.5.

2.2.9 Composite tissue blocks for immunohistochemistry

Tissue blocks identified for immunohistochemistry were prepared as follows:
The abnormal area within the tissue section was identified and marked on the H&E-stained slide with a permanent marker pen. Correlation with the paraffin wax-embedded block was made and the paraffin wax-embedded block marked in the same area, again with a permanent marker pen. The abnormal area within the paraffin block was excised with a tissue punch, 2mm diameter. Composite blocks containing up to 20 separate cases were prepared by embedding the identified abnormal tissues in paraffin wax, together with similar 2mm punch biopsies of tonsil, placenta, kidney and liver. These tissues acted both as orientation guides and as internal staining controls.

Histological diagnosis	Morphological characteristics
plasma cell deciduitis	plasma cells within placental decidua (61)
eosinophilic inflammation	small lymphocytes and eosinophils associated with chorionic plate vessel(s) (62)
subacute chorionic plate inflammation	small lymphocytes and/or histiocytes within chorionic plate (64)

Table 2.3: Diagnostic characteristics of additional histological categories for sub-analysis

Micro-anatomical location of inflammation	Morphological characteristics
free membranes	collections of polymorphonuclear leukocytes within amnion/chorion, > 5 per cluster
umbilical cord	migration of polymorphonuclear leukocyte(s) within endothelium, muscularis or Wharton's jelly
subchorionic fibrin	collections of polymorphonuclear leukocytes within subchorionic fibrin, > 5 per cluster
chorionic plate	migration of polymorphonuclear leukocyte(s) within endothelium or muscularis

Table 2.4: Subclassification of cases showing histological features of ascending infection

Variant of VUE	Morphological characteristics
focal low-grade VUE	clumped clusters of chorionic villi infiltrated by small lymphocytes and histiocytes, < 10 chorionic villi per cluster, changes present on one slide only
multifocal low-grade VUE	clumped clusters of chorionic villi infiltrated by small lymphocytes and histiocytes, < 10 chorionic villi per cluster, changes present on > one slide
patchy high-grade VUE	clumped clusters of chorionic villi infiltrated by small lymphocytes and histiocytes, >10 chorionic villi per cluster, changes present on one slide only
diffuse high-grade VUE	clumped clusters of chorionic villi infiltrated by small lymphocytes and histiocytes, >10 chorionic villi per cluster, changes present on > one slide

Table 2.5: Subclassification of cases showing villitis of unknown etiology

2.2.10 Immunohistochemical staining

The primary antibodies applied to selected cases were as summarized in Table 2.6 below

Primary antibody	Raised In	Dilution	Antigen retrieval	Supplier	Control
CD3	Mouse	ready-made	heat 20 mins pH9	Leica	tonsil
CD20	Mouse	1:1000	heat 20 mins pH9	DAKO	tonsil
HLADR	Mouse	1:3000	heat 20 mins pH6	DAKO	tonsil
CD68	Mouse	1:200	enzyme 1 - 10 mins	DAKO	tonsil
C4d	Rabbit	1:40	heat 20 mins pH9	Biomedical	tonsil
cleaved caspase 3	Rabbit	1:50	heat 20 mins pH6	Cellsignalling	appendix
ICAM	Mouse	1:25	heat 10 mins pH6	Leica	tonsil
P selectin	Mouse	1:100	heat 20 mins pH6	Leica	tonsil
Fox P3	Mouse	1:50	heat 20mins pH6	AbCam	tonsil

Table 2.6: primary antibodies, dilution, antigen retrieval, supplier and control tissue for immunohistochemical staining.

The secondary antibody used was a DAB-conjugated rabbit antimouse antibody used at a dilution of 1:100.

Immunohistochemical staining was carried out in accordance with widely published protocols (140).

2.2.11 Scoring of immunohistochemistry

Villous area:

The area of placental villi included in each 2mm punch biopsy was calculated by use of ImageJ software (139). Each punch biopsy (within a microarray of up to 20 cases) was

photographed, with a graticule scale also photographed at the same magnification as the cases.

The photomicrographs were analysed as follows (see Figure 2.1):

1. Set scale: analyze>set scale>measure known distance on photomicrograph of scale included in photograph.
2. Known distance entered, calibrated with given number of pixels, scale set.
3. Open photomicrograph of case. Areas of non-villous tissue (fibrin, accumulations of red blood cells, included chorionic plate etc) all identified. Freehand tool selected, outline of each non-villous area made: edit>cut.
4. Resulting image (with villous tissue only) modified to 8-bit image: image>type>8-bit. 8-bit image made binary: process>binary>make binary.
5. Resulting black and white image reviewed: if villi closely packed: process>binary>watershed.
6. Area of villi calculated: analyze>analyze particles.

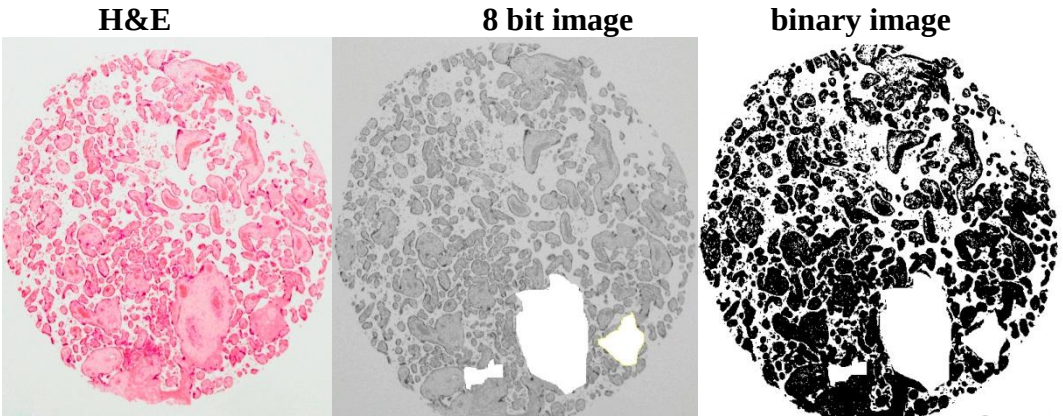


Figure 2.2: Processing of image for image analysis: H&E image → 8 bit image → binary image (x 2 objective).

Immunohistochemically stained slides were scored by recording positively staining cells/membranes in each section with a tally counter and dividing the number of positive counts by the villous area calculated in mm^2.

2.2.12 Systematic reviews

The systematic review approach taken to support this study was based on a standardised Cochrane review systematic review model (141). The methodology is described in detail in Chapter 4.

In summary, specific criteria were set for inclusion and advanced searches of MEDLINE and EMBASE were undertaken. Review of abstracts was independently undertaken by FAJ and NJS. Hand searching of relevant journals, searching of returned manuscript reference lists and direct email/postal approach to authors of conference abstracts was also undertaken. Data were extracted onto standardised forms.

Evaluation of study quality preceded data extraction. Analysis of the data were carried out using RevMan software (Review Manager v5.1.6). (142).

2.2.13 Statistical analysis of data

The statistical techniques used in this study are derived from printed material and software supplied in courses run by the University of Cambridge Centre for Applied Medical Statistics (143) and the University of Nottingham Systematic Review Course (142, 144). A standard statistical text was also referred to (145). Statistical analysis was undertaken with Microsoft Excel, GraphPad software (146), RevMan software (142) and the open source epidemiological web resource, OpenEpi (147).

Chi^2 test, Fisher's exact test, Mid-p exact, Mantel-Haenszel test

These tests analyse data presented in a contingency table, an example of which is given below

	Observation X	Observation Y	Total
Group A	200	250	450
Group B	100	450	550
Total	300	700	1000

Table 2.7: Example of contingency table, with illustrative values

Chi^2 testing and Fisher's exact test measure the significance of association between the two classifications in the table (observation X and observation Y).

Both the chi^2 test and Fisher's exact test analyse categorical data (eg placental finding present - Group A, or absent - Group B) with numerical data (numbers in each group). The counts are presented in a contingency table with analysis of counts in the context of predetermined level of significance and degrees of freedom (based on the numbers of columns and rows). Chi^2 testing makes assumptions about the sampling distribution of the

population, and so Fisher's exact test (which becomes unbalanced with large populations) is used with smaller populations. By convention, Fisher's exact test is used when a cell in the contingency table contains a value of 5 or less. Many statistics programmes provide both Fisher's exact and mid P exact values. Fisher's exact test is "conservative" – it requires more evidence than is necessary to reject a null hypothesis. Mid P is more "powerful", is better suited to larger populations, and delivers a p value that is closer to the p value for entirely uncorrected data. In this study, the possible conflict between small numbers (in individual subgroups) and large numbers (the whole study) is addressed by examining, where appropriate, both Fisher's exact and mid P values.

The Mantel-Haenszel test is used in meta-analysis, providing a pooled odds ratio (qv) across independent studies, by analyzing data entered in multiple 4x4 contingency tables. It thus tests whether the overall degree of association is significant. When combined with a chi^2 test, data can be assessed for equality as well as a pooled odds ratio.

D'Agostino-Pearson normality test

A completely normally distributed population is expressed graphically as a bell-shaped curve, symmetrically distributed about the mean. The spread depends on the variable under study, but ~ 68% of observations lie within 1 standard deviation of the mean, and 95% of observations lie within 2 standard deviations of the mean. A data set may be tested for "skewdness" and deviation from normal distribution with a normality test formula, which calculates the variation of each value from the value expected with a Gaussian distribution, and computes a single p value from the sum of these discrepancies.

Mann-Whitney test

The Mann-Whitney test compares the median values of two groups – with a small p value, the null hypothesis that there is no difference between the median values of two groups can be rejected. A large p value does not imply that the medians are the same, but provides no statistical evidence that they differ. Gaussian distribution is not necessary for this analysis.

Odds ratio and 95% confidence interval

An odds ratio is commonly expressed as a statistical "best guess" as to the size and direction of the effect of an experimental intervention compared to a control intervention. In this study, this concept is adapted in that the "intervention" takes the form of a specific

placental finding with the control group formed by those cases in the study group not showing this finding.
The odds ratio is presented as a single number – a point estimate. A positive odds ratio implies a positive association with a given clinical outcome, while a negative odds ration implies a negative (or protective) association with a given clinical outcome. The odds ratio is, however, expressed in the context of a 95% confidence interval. Very wide confidence intervals imply that the true effect is unclear, and a confidence interval which crosses zero implies that there is no clear evidence for a significant odds ratio in either direction.

One way ANOVA and Bartlett's test

This statistical test assesses the equality of three or more means, with the null hypothesis that the population means are equal. This test assumes normal distribution, and will identify at least one mean which is different – although not where the difference lies. The ANOVA test also assumes equal variance (the spread of values from the mean) within each population, but this can be confirmed by applying Bartlett's test to the data sets.

P values (one tailed and two tailed)

The p value is a numerical expression of the outcome of a null hypothesis (H_0) test. For example, in this study, observed placental findings are analysed in the context of clinical outcome, generating the null hypothesis that placental finding A is not associated with clinical outcome B. Rejection of the null hypothesis requires a p value less than a specific value, conventionally < 0.05 – implying that there is a probability of 0.95 that rejection of the null hypothesis is the correct decision. In this study, given the large number of calculations undertaken, p values around 0.05 are carefully reviewed.

The p value generated from the testing of any null hypothesis can be expressed as one-tailed or two-tailed. One-tailed p values are used in a context only where there is pre-existing statistical evidence for an effect that is unidirectional. In this project, a one-tailed p value would imply, for example, that placental finding A is already known (from pre-existing analysis of the project data) to be positively associated with clinical outcome B. This does not apply to the data in this project and so two-tailed p values, which test for statistical significance in both directions, are used throughout.

Spearman's rank correlation coefficient

Spearman's rank correlation coefficient measures the strength of association between two ranked variables. This test applies to monotonic variables: where one variable increases, a second increases/decreases. The value generated, r_s, lies between +1 (perfect association), 0 (no association) and -1 (perfect negative association). The data do not have to conform to a particular pattern of distribution for this analysis (non-parametric).

T test

The t test compares the means of two groups for statistical significance, while taking into account the spread of the data (standard deviation) and number of subjects in each group (degrees of freedom).

z scores

z scores standardize measurements from different groups, by transforming raw values to a score expressing the standard deviation from the mean for each data value. Initial calculation of the mean and standard deviations for each data group are thus required. The resulting values are often thus more informative than comparison of means between groups, as each data value is indexed.

2.3 CONCLUSIONS

- Recruitment protocols drawn up
- Clinical data recorded on a password protected NHS Trust server[1]
- Placentas examined in accordance with a standardized protocol[1]
- Analysis of placental eccentricity (shape) and cord insertion undertaken[1]
- Computer based analysis of photographs showing placental infarction undertaken
- Tissue blocks from the prosected placentas processed to paraffin[2]
- Haematoxylin and eosin[2] and immunohistochemical staining[3] of tissue sections undertaken
- Diagnostic criteria for a range of placental lesions drawn up, with two rounds of reporting[4]: the first in accordance with current clinical reporting protocols, the second to identify all possible histological lesions
- Computer based analysis and scoring of immunohistochemical slides undertaken
- Systematic review methodology reviewed and adapted for histopathology-based research
- Statistical tests selected for analysis of project data

1. Collection of clinical data, analysis of placental eccentricity and cord insertion site and approximately 50% of placental prosection was undertaken by Dr Sangeeta Pathak, Clinical Research Fellow, Department of Obstetrics and Gynaecology, Addenbrooke's Hospital.
2. Tissue block processing, cutting and H&E staining were carried out by members of the biomedical science team, Addenbrooke's Hospital.
3. Immunohistochemical micro-array tissue blocks and immunohistochemical staining was undertaken by Ms Rebecca West, biomedical scientist, Great Ormond Street Hospital.
4. The initial round of reporting was undertaken jointly between Dr F Jessop and Professor NJ Sebire.

CHAPTER THREE: BASELINE CLINICAL AND PLACENTAL CHARACTERISTICS OF STUDY POPULATION

Summary:

- Demographic attributes and clinical characteristics of subjects
- Obstetric and neonatal outcomes
- Placental macroscopic findings
    - Cord coiling index
    - Macroscopically identified infarcts
- Placental histological findings
    - No significant abnormality
    - Features of ascending genital tract infection
    - Features of chronic placental underperfusion
    - Villitis of unknown etiology
    - Fetal thrombotic vasculopathy
    - Chronic histiocytic intervillositis
    - Massive perivillous fibrin deposition
    - Intervillous thrombus
    - Chorangiosis
    - Chorangioma

3.1 INTRODUCTION

In this study, the target population was all women with an ongoing singleton pregnancy during the recruitment period 2007-2008. The study population was thus formed by those subjects available to the researchers involved in this study – ie women booking for delivery in the Rosie Hospital, Cambridge. Finally, the sample population constituted those women giving informed written consent to inclusion in the study during the recruitment period.

Sampling error was controlled for as much as possible by recruiting a large number of subjects to the study. On completion of the study, retrospective comparisons with incidence of the clinical outcomes of interest in this study were made with data available within published literature.

The study took the form of an unselected prospective cohort study. The cohort study is an effective tool in the identification of risk factors causing a particular outcome and was thus the most appropriate form of study (compared to, for example, a case control study) to answer the specific research question posed by this particular study: to determine whether any of a range of different macroscopic and microscopic findings within the delivered placenta are associated with specific obstetric and neonatal outcomes. Drawbacks of the cohort study include the relatively high financial and professional resources required for successful completion, and the difficulties in studying specific conditions with a low incidence within the target population.

3.2 METHODS

Consecutive subjects were recruited from their contact with obstetric services in the Rosie Hospital. Posters were placed in patient areas (eg antenatal clinics, ultrasound department, wards and delivery unit). Excluded subjects were those unable to give informed consent, those with multiple pregnancy or those found not to have an ongoing pregnancy on booking. Women were given study information leaflets on presentation at booking, with consent for participation obtained largely on presentation to the delivery unit.

There was thus a relative bias in recruitment to relatively uncomplicated term pregnancies: those women presenting in preterm labour, or with obstetric emergencies, tended to be lost to recruitment given the urgency of dealing with the presenting clinical problem. The

advantage to this bias is that the data collected represent the findings in a largely healthy, largely term population, allowing study of specific placental lesions biased away from adverse clinical outcomes. The study was based on consecutive recruitment – all those subjects consenting to involvement were included.

Data entry was made onto secure servers within the Departments of Obstetrics and Histopathology, with the databases united only on completion of all data collection: until that point, all researchers involved in the study were blinded to all findings within the study other than their own individual observations.

3.3 STATISTICAL ANALYSIS

The initial data sets are given below. Data are grouped according to type – discrete or continuous variables etc. Limited statistical analysis has been carried out on the initial data sets: where appropriate, percentage calculations have been performed, mean values are presented with standard deviations, and infarct data are presented as a frequency histogram.

3.4 RESULTS

3.4.1 Demographics

The demographics and clinical characteristics of the study population are given in tables 3.1, 3.2 and 3.3. The population studied, with a bias towards women presenting close to term in uncomplicated labour as noted above, was that of a predominantly low-risk Caucasian population with a smoking rate of 9%.

Study population	1119
Mean age	31 (SD 5.75)
Primiparous subjects	535
Caucasian subjects	1013
Pre-existing (essential) hypertension	5
Smokers	102
Type 1 diabetes	4

Table 3.1 Baseline population characteristics of 1119 subjects

3.4.2 Clinical outcomes

The clinical outcomes selected for the study included a range of known and potentially adverse outcomes, to ensure that as many associations as possible between possibly significant macroscopic/microscopic placental lesions and clinical compromise were detected. For example, the threshold for neonatal acidosis was set at pH 7.2 rather the more often quoted pH 7.0 (148), to maximize the detection of marginally compromised neonates. The clinical outcomes are summarized in tables 3.2 – 3.4.

Clinical outcome	**Percent (number of cases)**
Pregnancy-induced hypertension	2.4 (27)
Pre-eclamptic toxaemia of pregnancy	2.1 (24)
Antepartum intra-uterine growth restriction	1.1 (12)
Suspected maternal chorioamnionitis	0 (0)
Clinical abruption	0.08 (1)
Spontaneous onset of labour	63 (710)
Induced onset of labour	20 (218)
Elective lower segment caesarian section	14 (157)
Mode of onset of labour not recorded	3 (34)
Spontaneous vertex delivery	63 (705)
Assisted vaginal delivery	12 (130)
Emergency lower segment caesarian section	11 (127)
Gestation ≥ 37 weeks	97 (1085)
Gestation ≤ 37 weeks	3 (34)

Table 3.2: Obstetric outcomes in 1119 singleton deliveries

Mean birth weight, g	3485 (SD 543)
Mean birth weight, boys, g (n=596)	3460 (SD 486)
Mean birth weight, girls, g (n=523)	3510 (SD 510)
Mean Apgar score @ 1 minute	9 (SD 0.88)
Mean Apgar score @ 5minutes	10 (SD 0.59)

Table 3.3: Neonatal outcomes (means and standard deviations) in 1119 singleton deliveries

Clinical outcome	**Percent (number of cases)**
Birth weight < 10th centile	9.5 (106)
Birth weight < 10th centile, boys	4.2 (47)
Birth weight < 10th centile, girls	5.3 (59)
Neonatal acidosis (pH < 7.2)	7.3 (82)
Apgar score < 7 @ 1 minute	3.0 (34)
Apgar score < 7 @ 5 minutes	0.3 (3)
Suspected neonatal sepsis	3.7 (42)
Admission to neonatal intensive care	5.9 (67)

Table 3.4: Neonatal outcomes (percentage and number of cases) in 1119 singleton deliveries

3.4.3 Placental macroscopic findings

1082 placentas had sufficient attached cord (≥ 15cm) to allow calculation of the cord coiling index (number of complete turns/cm). The mean cord length of these 1082 placentas was 43cm (SD=13) and the mean cord coiling index 0.20 (SD=0.09).

43 placentas (3.8% of the cohort) had macroscopically visible infarcts, as represented in Figure 3.1.

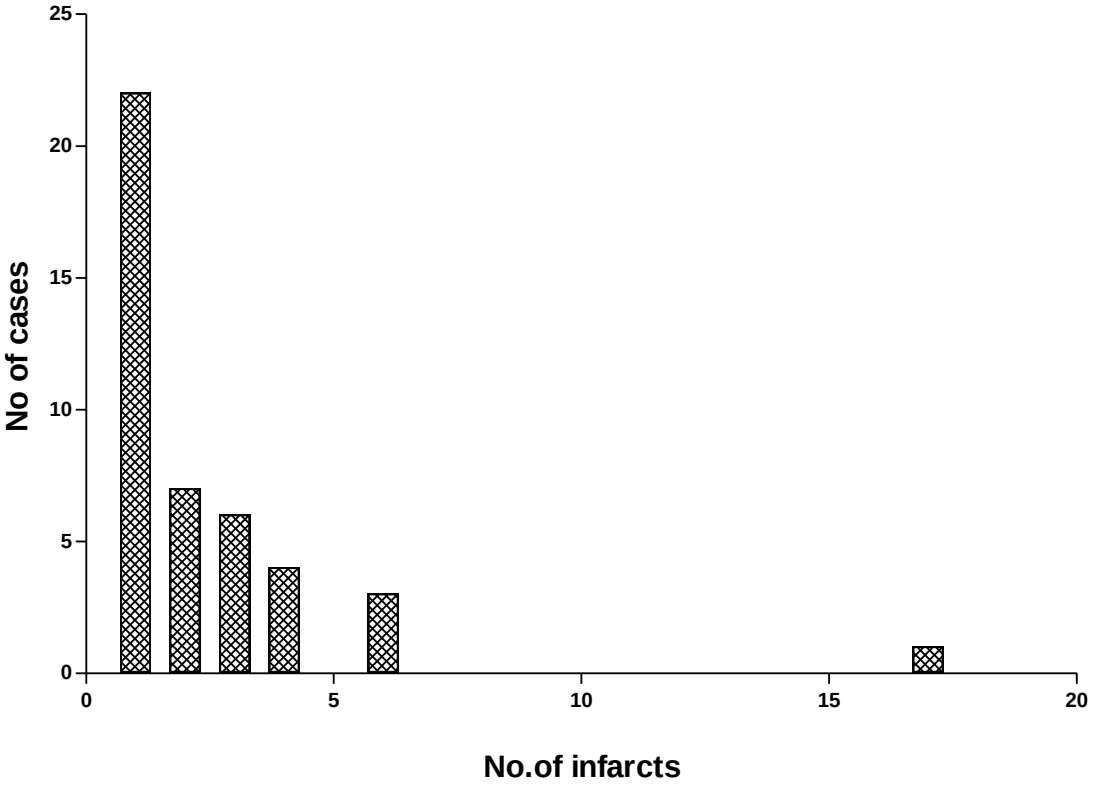


Figure 3.1. Frequency histogram detailing number of infarcts/case in 43 cases of macroscopically identified infarction

A further 12 cases of infarction were recognised on histological examination. In total, therefore, 55 cases (4.9% of the cohort) of placental infarction, whether macroscopically or microscopically identified, were diagnosed.

3.4.4 Placental microscopic findings

The histological reporting of the 1119 delivered placentas took place over two rounds, as described in Chapter 2. Initially, cases were categorized as per currently recognized reporting protocols. In particular, low-grade inflammatory lesions were disregarded. This allowed initial analysis of clinical outcomes in relation to current histological reporting protocols (15, 149)

For this study, the objective was to identify all possible placental histological variants, whether previously considered to be pathological or not, in order to define specific histological subgroups and relate them to clinical outcomes. Further analysis of the subgroups identified by dropping the reporting threshold to any possible lesion, rather than any lesion considered to be significant in the context of current clinical practice, added a further 128 cases with potentially abnormal histology. The single largest category (67 cases) was low-grade acute inflammatory change, with additional cases represented by low-grade villitis of unknown etiology (23 cases) and various inflammatory lesions of uncertain pathological significance (subacute chorionitis, plasma cell deciduitis, eosinophilic vasculitis) (24 cases).

Histological diagnosis	Percent (number of cases)
no significant histological abnormality.	71 (792)
features of ascending genital tract infection.	11 (121)
features of chronic placental underperfusion, including pre-eclampsia *	7.1 (79)
villitis of unknown etiology	3.7 (41)
fetal thrombotic vasculopathy *	0.98 (11)
features of acute abruption *	0.09 (1)
chronic histiocytic intervillositis	0.27 (3)
massive perivillous fibrin deposition *	0.45 (5)
intervillous thrombus *	4.7 (53)
chorangiosis	0.80 (9)
chorangioma *	0.36 (4)

Table 3.5 First round of histological reporting (50% FAJ, 50% NJS)
* Sampling protocols were drawn up to ensure that lesions which rely on correlation with the placental macroscopic findings were reliably and representatively sampled for histological assessment, please see Appendix 4.

Histological diagnosis	Percent (Number of cases)
no significant histological abnormality.	58 (664)
features of ascending genital tract infection.	17 (188)
features of chronic placental underperfusion, including pre-eclampsia	7.3 (82)
villitis of unknown etiology	5.7 (64)
fetal thrombotic vasculopathy	1.2 (13)
features of acute abruption	0.80 (9)
chronic histiocytic intervillositis	0.27 (3)
massive perivillous fibrin deposition	0.71 (8)
intervillous thrombus	4.7 (53)
chorangiosis	0.80 (9)
chorangioma	0.18 (2)
plasma cell deciduitis	0.54 (6)
eosinophilic inflammation	0.62 (7)
subacute chorionic plate inflammation	0.98 (11)

Table 3.6: Second round of histological reporting (all normal from 1st round of reporting, FAJ)

Micro-anatomical location of inflammation	Number of cases
free membranes only	12
mixed chorionic plate/free membranes	16
chorionic plate	28
subchorionic fibrin	68
umbilical cord	64

Table 3.7: Subclassification of cases showing histological features of ascending infection (n= 188)

Number of inflamed umbilical cord vessels	Number of cases
1	48
2	9
3	7

Table 3.8: Subclassification of funisitis (n=64)

Variant of VUE	Number of cases
focal low-grade VUE	23
multifocal low-grade VUE	21
patchy high-grade VUE	11
diffuse high-grade VUE	9

Table 3.9: Subclassification of cases showing villitis of unknown etiology (n=64)

3.5 DISCUSSION

Clinical Outcomes

Studies detailing clinical outcomes in unselected low-risk populations delivering at term are relatively rare within the literature. When compared with available statistics for the more commonly reported outcomes in low-risk deliveries in England (150, 151) and studies published in Switzerland (152) and Canada (153), the rates of obstetric and neonatal events in the present Cambridge study fall into the range of these previously published studies (see Table 3.10). There is clearly a degree of variation, some of which may be attributable to differences within populations and clinical management regimes.

Study (reference)	Study Period	No of subjects	Interventional deliveries (%)	Apgar score < 7 @ 1 minute (%)	Apgar score < 7 @ 5 minutes (%)	Neonatal acidosis (5)
NHS Hospital Episode Statistics (150)	2010 - 2011	668195	26	_	_	_
Birthplace in England (151)	2008-2010	64538	15	_	_	_
Switzerland (152)	2000-2001	3395	23	_	3	10.2
Canada (153)	1998 – 1999	1314	22	13.5	0.9	_
Cambridge	2007 – 2008	1119	23	3	0.3	7.3

Table 3.10: Clinical outcomes in comparable studies

Birthweights for completed gestational weeks in the study cohort are given in comparison to United Kingdom national statistics (150) in table 3.11

Completed gestational weeks	Cambridge cohort mean weight (g)	National cohort mean weight (g)
36	2760	2712
37	3001	2956
38	3286	3180
39	3515	3340
40	3541	3483
41	3702	3630
42	3951	3710

Table 3.11: Birthweights for completed gestational weeks, study cohort vs national cohort

This study design also included a more extended range of clinical outcomes, with the objective of identifying all possible instances of suspected adverse outcome. The range of outcomes studied introduced occasional anomalies. A zero incidence of clinically suspected maternal chorioamnionitis was recorded. While this in part may be accounted for by the low-risk, predominantly term population studied, it is also apparent that in retrospect the clinical inclusion criteria for a diagnosis of clinical chorioamnionitis (two of maternal pyrexia, leucocytosis, offensive discharge or abdominal tenderness) may have

been overly rigid. Cases of suspected neonatal sepsis (infants with clinical history/findings sufficient to initiate blood cultures or antibiotic treatment) thus became the preferred indicator of infection, in this study, for correlation with acute inflammatory placental lesions.

Macroscopic findings

The mean and range of the cord coiling index in the study population in this project was similar to those reported for American (154) and Dutch (155) populations. Cord coiling indices in relation to clinical outcomes are considered in Chapter 4.

Studies reporting rates of placental infarction in low-risk term placenta are, perhaps surprisingly, rare – extracting data from the published literature largely comes in the form of control groups taken from case control studies of small for gestational age infants. For the few studies of reportedly low-risk pregnancies delivering at term, the rates of placental infarction are as given in table 3.12.

Study (reference)	**Study Period**	**No of subjects**	**Cases of placental infarction (%)**
Becroft (20)	1995-1997	529	11.7
Salafia (34)	2000 – 2001	179	10.1
Stanculescu (156)	2008-2010	474	19.2
Blair (33)	1980 – 1985	491	2.0
Cambridge	2007-2008	1119	4.9

Table 3.12: Comparison of rates of placental infarction amongst studies

It is notable that the rates of placental infarction are quite variable amongst studies. The reasons are unclear. In the Cambridge study, macroscopically identified infarcts were all confirmed histologically, and additional cases were diagnosed histologically, giving little scope for systematic failure to recognize true infarction in the present series. The differences overall may relate both to true differences amongst populations rather than under-recognition in this study and differences in criteria for diagnosis of an infarct.

Microscopic findings

One of the principal questions of this study was to determine the clinical significance of all acute and chronic inflammatory lesions, particularly when analysed in subgroups, no matter how apparently insignificant the lesion appeared histologically. For this reason, rapid reporting of placental histological sections in line with current reporting protocols

was undertaken in the first instance, with a subsequent rescreen of all negatively reported slides in a second round of reporting. The inclusion in this study of all possible lesions, however minimal, does make comparison of the incidence of other inflammatory lesions in other studies difficult.

For acute inflammatory changes, small numbers of studies exist, reporting variable levels of acute inflammation in term placentas, ranging from 10% to 17% in n=20 and n=661 studies of high-risk deliveries (38, 157) to 35% in an n=195 study of low-risk deliveries (158). The definitions of histological chorioamnionitis in these studies are, however, variable, and are thus of limited value when attempting to extrapolate to different populations. For this reason, in this study, previously proposed histological staging/grading systems for chorioamnionitis (36) were not considered to be useful. Microanatomical localization of acute inflammatory lesions was preferred in the present study, representing a descriptive, simplified approach to classification which could relatively easily be correlated with specific clinical outcomes. The Cambridge cohort study has thus generated very useful baseline data, describing the incidence of acute inflammation in low-risk term placentas, which is likely to serve as a reference point within the literature in this field. Chapter 6 describes the results of further sub-analysis and immunohistochemical characterization of the acute inflammatory lesions in this study.

The rate of chronic villous inflammatory lesions – of any grade - in this study was 5.7%. When set against the rates in previously reported studies, this rate is comparable, as shown in Table 3.12

Study (reference)	**Study Period**	**No of subjects**	**Cases of VUE (%)**
Russell (159)	1976-1978	7505	7.6
Knox (50)	1979 – 1980	1000	13.6
Becroft (160)	1995 – 1997	529	11.7
Vedmedovska (161)	2007 – 2008	50	6
Cambridge	2007 – 2008	1119	5.7

Table 3.13: Comparison of rates of villitis of unknown etiology (VUE) amongst studies

These findings are further analysed, together with immunohistochemical characterisation, in Chapter 7.

3.6 CONCLUSIONS

- 1119 low-risk subjects recruited, delivering at or near to term.
- Clinical outcomes and rates of potentially pathological placental lesions within the present study group compared to available data within the literature
- In the Cambridge cohort:
    - Rate of over/undercoiling of umbilical cords approximately 20%
    - Rate of macroscopic placental infarction = 3.8%. An additional 1.1% of cases found to have infarction on microscopic examination.
    - Rate of acute histological placental inflammation (histological features of possible ascending genital tract infection): 11% when current routine histological reporting protocols applied; 17% with all cases showing any evidence of acute histological inflammation included.
    - Rate of unexplained chronic placental parenchymal inflammation (chronic villitis of unknown etiology) = 3.7% when current routine histological reporting protocols were applied. Rate = 5.7% with all cases showing any evidence of chronic histological villous inflammation included.
- These lesions (over/undercoiled umbilical cords, placental infarction, acute histological inflammation and chronic villous inflammation) selected for further sub-analysis and correlation with clinical outcome in later Chapters (4-7).

CHAPTER 4: UMBILICAL CORD OVERCOILING AND UNDERCOILING: CLINICAL OUTCOMES IN AN UNSELECTED POPULATION AND SYSTEMATIC REVIEW

Summary:

- Calculation of coiling index in placentas with umbilical cord length > 15cm
- Recording of five adverse neonatal outcomes in infants with umbilical cord length > 15cm.
    - Interventional delivery
    - Birthweight < 10^{th} centile
    - Apgar score < 7 at one minute of age
    - Neonatal acidosis (pH < 7.2)
    - Admission to the neonatal intensive care unit
- Systematic review
    - Study inclusion criteria
    - Literature search strategies

4.1 INTRODUCTION

The human umbilical cord has an average length of around 50 cm, with approximately 11 helical or spiral turns, parallel to the course of the vessels within. Assessment of the number of twists per unit length was defined in 1994 as the umbilical cord coiling index (17). While mechanical cord lesions (eg tight nuchal cord, cord prolapse, true cord knots) are incontrovertibly associated with adverse ante-partum or intra-partum events (162-164) there has been, for some time, considerable and ongoing interest within the literature in the relationship between the umbilical cord coiling index (derived both sonographically and following delivery) and clinical outcomes (154, 155, 165-171).

Specific associations between variations in cord coiling outwith the presumed normal range and adverse perinatal events have been proposed in multiple studies over a number of years (18, 154, 155, 172, 173). The possible adverse outcomes identified are varied but very often those reported carry significant clinical implications: stillbirth, Apgar score < 7, intra-uterine growth restriction, fetal/neonatal acidosis and asphyxia. The topic remains controversial, with reservations expressed relating both to study design (174) and the likely clinical significance of reported positive associations (175). The mechanism(s) suspected of linking perceived abnormal umbilical cord coiling with adverse clinical outcomes are uncertain, but are commonly suggested to relate to fetal hypoxia, secondary to increased cord vulnerability to compression or kinking.

4.2 METHODS

This was an unselected prospective study, as discussed elsewhere. 1,082 delivered placentas had sufficiently long attached umbilical cords (>15 cm) to allow assessment of the coiling index. Placental examination was carried out either by FAJ or SP, with placental macroscopic examination carried out in accordance with previously published protocols (136, 176). Coiling index was calculated as described by Strong (17).

Markers of severely adverse clinical outcome in neonates include seizures, severe acidosis and death. For this study, in order to identify as many potentially adverse outcomes relating to variations in cord coiling index as possible a wider range of candidate adverse clinical outcomes were included – some were of a less severe nature, but still had the potential to identify fetuses/neonates with a degree of physiological compromise. These

clinical outcomes included interventional delivery, birthweight < 10 centile, Apgar score < 7 at 1 minute of age, neonatal acidosis (pH<7.2) and admission to the neonatal special care unit (previously reported in an initial cohort of 888 women with cord coiling studied in relation to placental shape) (24).

A standardised systematic review model was employed to undertake the review. The review was constructed with specific criteria summarised in Table 4.1. Advanced searches of MEDLINE and EMBASE were undertaken over July 2011 to March 2012; search strategies are summarised in Table 4.2. Hand searching of relevant journals, searching of returned manuscript reference lists and direct email/postal approach to authors of conference abstracts was also undertaken. The process of selection is summarised in Figure 2. Independent review of abstracts was undertaken by FAJ and Professor NJ Sebire, Great Ormond Street Hospital, London, with data extracted onto standardised forms: for each study, the minimum data required for inclusion of the study into the systematic review were: clearly recorded numbers of participants with hypo-coiled (<10 centile), normally coiled (10^{th} – 90^{th} centile) and hyper-coiled (>90^{th} centile) umbilical cords plus the numbers of participants from each group experiencing at least one of the clinical outcomes as set out in Table 4.3. Statistical analysis was carried out by constructing forest plots of events occurring within individual studies expressed as odds ratios plotted on a log scale. Statistical heterogeneity was examined by comparison of the Chi^2 value with the degrees of freedom (df). Risk of bias analysis and assessment of clinical heterogeneity was carried out as outlined in Cochrane review methodology (177).

types of study	prospective cohort retrospective cohort
participants	pregnant women with ongoing singleton pregnancy > 16 weeks umbilical cord coiling index recorded by ultrasound or following delivery
pathological event (exposure)	umbilical cord coiling <10^{th} centile or > 90^{th} centile
outcome measure recorded	Apgar score < 7 interventional delivery neonatal acidosis fetal growth < 10^{th} centile

Table 4.1: Criteria for considering studies for systematic review of umbilical cord coiling

MEDLINE	**EMBASE**
longitudinal OR cohort OR follow-up OR prospective OR retrospective OR case control	umbilical cord.mp or exp umbilical cord or umbilical artery.mp or exp umbilical artery or umbilical vein.mp or exp umbilical vein or umbilical vessel or exp umbilical vessel or
AND	AND
umbilical cord [Title/abstract] OR umbilical vessel [Title/abstract] OR umbilical vascular [Title/abstract] OR umbilicus [Title/abstract] OR "umbilicus" [Mesh] OR "umbilical veins" [Mesh] OR "umbilical cord" [Mesh] OR "umbilical arteries" [Mesh]	coil*.mp. or spiral*.mp. or twist*.mp. or helic*.mp. or helix.mp.
AND	AND
(coil*) OR (spiral*) OR (twist*) OR (helix*) OR (helic*)	exp cohort analysis/ or cohort.mp or exp retrospective study/ or retrospective.mp or prospective.mp. or exp prospective study or exp longitudinal study/ or longitudinal.mp. or exp case control study/ or case control.mp.

Table 4.2: Search strategies for systematic review of umbilical cord coiling

4.3 STATISTICAL ANALYSIS

Determination of mean values and standard deviations was carried out using standard Microsoft Excel software. RevMan software (142) was used to calculate odds ratios and the Chi^2 statistic. GraphPad software (La Jolla, California, USA, www.graphpad.com) was used to create the frequency histograms, perform the subsequent analysis for normality with the D'Agostino and Pearson normality test. OpenEpi software (178) was used to determine p values from 2 x 2 table analysis, and also odds ratios with confidence intervals. Fisher's exact test was preferred, given the small numbers of cases in some subgroups.

4.4 RESULTS

Cambridge cohort study

1082 delivered placentas from the study population had sufficient attached umbilical cord for assessment of the umbilical coiling index. Of this group, mean maternal age was 30.7 years (SD=5.7) and 519 were primiparous. The mean cord length was 43cm (SD=13) and the mean cord coiling index 0.20 (SD=0.09), with a range of 0 to 0.55. Gaussian distribution of cord coiling in our cohort of 1082 was confirmed by the D'Agostino and Pearson normality test (K2 4.208, p value 0.1219) carried out on a frequency histogram (see Figure 4.1).

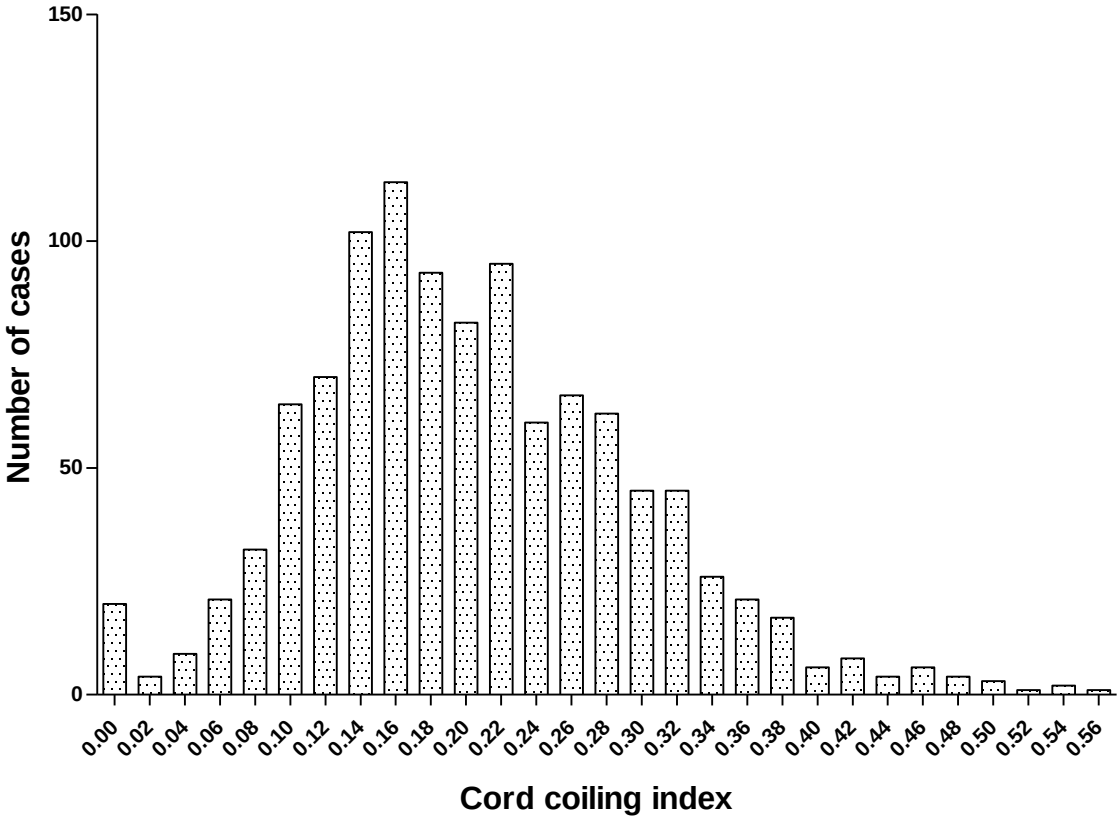


Figure 4.1. Frequency histogram of cord coiling index in 1082 cases with cords > 15cm.

Cases below the 10th and above the 90th centiles were subsequently identified, with 108 below the 10th centile ("hypo-coiled"), 866 between the 10th and 90th centile ("normally coiled") and 108 above the 90th centile ("hyper-coiled"). The mean coiling index of the hypo-coiled group was 0.06 (SD=0.03) with a range of 0 to 0.10. The mean coiling index of the hyper-coiled group was 0.38 (SD=0.06) with a range from 0.32 to 0.55.

No infants in this study presented with seizures and only two were found to have arterial blood pH < 7 at delivery, the latter group too small for meaningful statistical analysis. One death was recorded, a planned term delivery for anencephaly mapped onto a neonatal palliative care pathway.

The number of events in each group for the four specific clinical outcomes included in this study are given in Table 4.3. In view of the small numbers in some of the observed outcome groups, the data were analysed by creating 2x2 tables, with the outcome in either the <10th centile (hypo-coiled) group (n=108) or the >90th centile (hyper-coiled) group

(n=108) tested against the 10th-90th centile (n=866). Fisher's exact test and the mid P exact test was applied to these data. Odds ratios, with mid P exact lower and upper confidence limits, were also calculated. The p values and odds ratios generated from this analysis are given in Tables 4.5 and 4.6.

The mean birth weight and standard deviation for each of the three cord coiling groups were also calculated, and is summarized in Table 4.4. One way ANOVA testing was carried out, with the analysis given in Figure 4.2. This analysis shows equivocal variance amongst the groups, with a p value of 0.0446 showing significance not upheld by Bartlett's test for equal variances.

	hypo-coiled n=108 <10th centile	**hyper-coiled n=108 > 90th centile**	**normal n=866 10 – 90th centile**
Apgar < 7 @ 1min	4	3	27
interventional delivery (assisted vaginal/EMLSCS)	24	17	211
birth weight < 10th centile	10	15	75
admission to NICU	5	5	56
neonatal acidosis (arterial pH <7.2)	7	9	63

Table 4.3: Clinical outcomes in 1082 cases with > 15cm umbilical cord, stratified into hypo-coiled, hyper-coiled and normally coiled groups.

	hypo-coiled N=108	hyper-coiled N=108	normal N=866
mean birth weight (g) [SD]	3492 [478]	3371 [511]	3499 [505]
mean cord length (cm) [SD]	40 [13]	40 [15]	43 [13]

Table 4.4 : Mean birth weights and umbilical cord lengths in in 1082 cases with > 15cm umbilical cord, stratified into hypo-coiled, hyper-coiled and normally coiled groups.

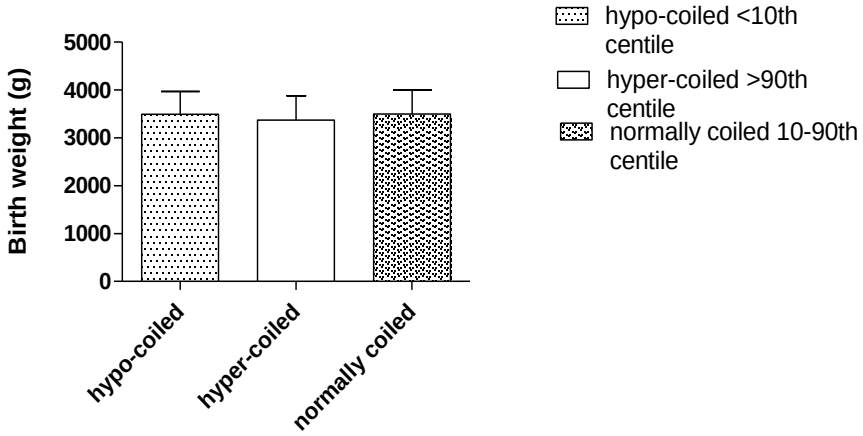


Figure 4.2. Analysis of birth weight variance amongst cord coiling groups: <10th centile, 10th -90th centile and >90th centile.

	Fisher's exact test (2-tail)	mid P exact test (2-tail)	odds ratio (95% confidence interval, mid P exact)
Apgar < 7 @ 1min	0.91	0.71	1.20 (0.35,3.25)
interventional delivery (assisted vaginal/EMLSCS)	0.72	0.63	0.88 (0.54,1.45)
BW< 10th centile	0.94	0.81	1.08 (0.51,2.09)
admission to NICU	0.62	0.48	0.70 (0.24,1.68)
neonatal acidosis (arterial pH <7.2)	0.95	0.80	0.88 (0.36,1.90)

Table 4.5: Statistical analysis of clinical outcomes, hypo-coiled cords (n=108) vs normally coiled cords (n=866)

	Fisher's exact test (2-tail)	mid P exact test (2-tail)	odds ratio (95% confidence interval, mid P exact)
Apgar < 7 @ 1min	>0.99	0.90	0.89 (0.21,2.7)
interventional delivery (assisted vaginal/EMLSCS)	0.05	0.04	0.58 (0.33,0.98)
BW< 10th centile	0.06	0.04	1.7 (0.91,3.03)
admission to NICU	0.62	0.48	0.70 (0.24,1.68)
neonatal acidosis (arterial pH <7.2)	0.40	0.33	1.16 (0.53,1.68)

Table 4.6: Statistical analysis of clinical outcomes, hyper-coiled cords (n=108) vs normally coiled cords (n=866)

Systematic review

Six published studies were included in the systematic review, together with the current cohort, with the process of selection summarized in Figure 4.3. The characteristics of the included studies are given in Table 4.7. No studies providing specific data relating to admission to neonatal intensive care were identified and so this outcome was not studied further in the context of systematic review.

study name (reference)	antepartum/ postpartum	study type	cohort year	number of subjects
Predanic (179)	antepartum	prospective	2003	294
Kashanian (170)	postpartum	prospective	2003-2004	699
Rana (171)	postpartum	prospective cohort	1994	508
de Laat 2006a (155)	postpartum	prospective cohort	2002-2003	885
de Laat 2007 (173)	postpartum	retrospective cohort	1998-2002	565
Chitra (180)	postpartum	prospective cohort	nk	1000
Cambridge	postpartum	prospective cohort	2007-2008	1082

Table 4.7: Characteristics of studies included in systematic review of clinical outcomes in cord coiling index variation.

Statistical heterogeneity – assessed by comparing the Chi^2 statistic with degrees of freedom, see Figures 4.4 to 4.11 – was striking, particularly in the analyses of hypo-coiling and hyper-coiling in relation to neonatal Apgar score <7 and interventional delivery. Overall, the Chi^2 statistic was less than degrees of freedom in only one group for each analysis of hyper- and hypo-coiled cords.

Clinical heterogeneity of studies was also judged to be high, with studies originating from the United Kingdom, the Unites States, the Netherlands, India and Iran and thus likely to include different baseline populations with different health care systems. Notably, the study closest in design to the Cambridge cohort study – that of Chitra et al – is likely to have been carried out on a substantially different clinical group: the results reported by Chitra et al include a low birth weight rate approaching 25%, substantially different to the Cambridge cohort rate of just under 10%. The number of low birth weight infants in the

Chitra cohort may also have confounded the recording of Apgar scores, which are more likely to be low in low birth weight infants (181).

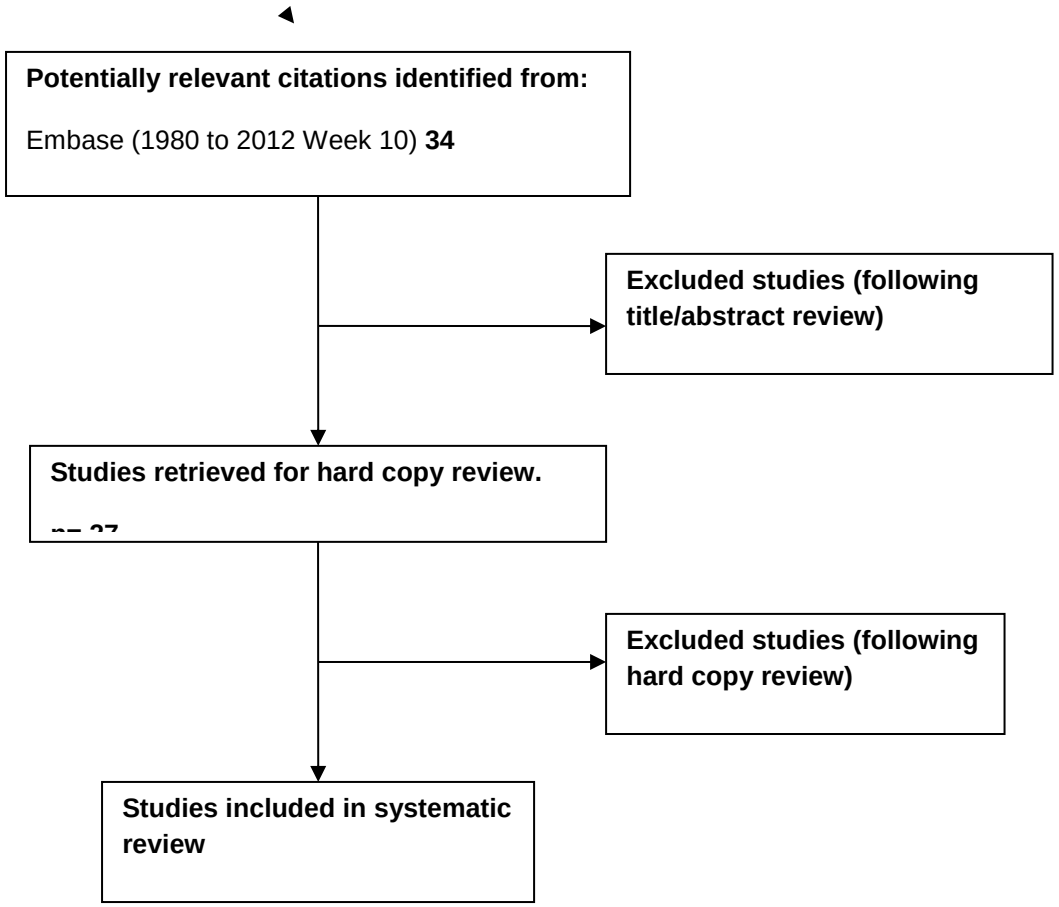
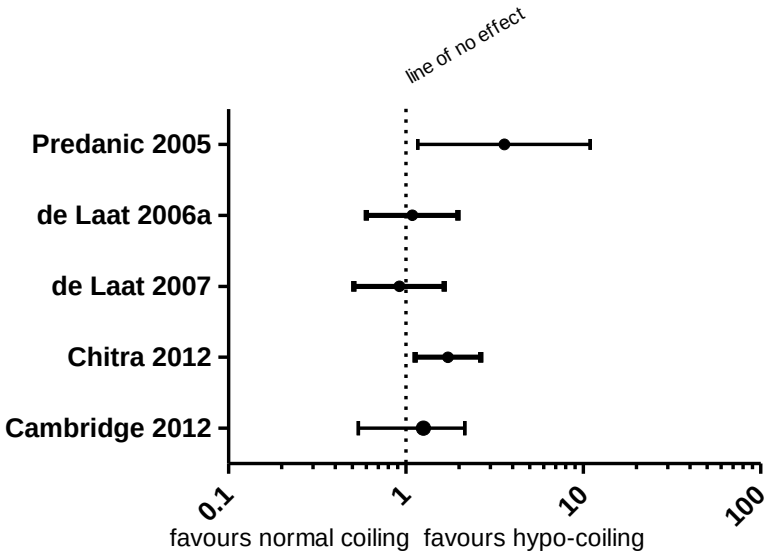


Figure 4.3: Outcomes of literature searches carried out for umbilical cord coiling systematic review.

For all studies included in the systematic review, including our own data, descriptive risk of bias analysis was carried out. The risk of reporting bias (unpublished negative data) in this systematic review is unknown, given that, to date, the cord coiling studies available in the literature report at least some positive findings. For all included studies, the risk of bias from attrition (variations in withdrawal from the study between groups) and detection (issues related to blinding of researchers) was judged to be low. For the studies by de Laat et al (155, 173), the risk of selection bias was high, with a population pre-selected for placental histological examination on clinical grounds and therefore likely to be high-risk with adverse outcomes. The risk of performance bias (variations in clinical care) was unclear, given the variations within intra-partum care across different countries (182). There was also high-risk of selection bias in the population included in the study by Rana et al (171), given that recruitment occurred in a high-risk perinatal centre. The risk of performance bias in this study was low. The risk of bias (selection and performance) within the Kashanian (170) and Chitra (180) studies was judged to be unclear. The Predanic (179) study had an unclear risk of bias for selection, given that 294 out of a baseline population of 425 met the study inclusion criteria of adequate ultrasound images and clinical history. The Cambridge cohort had an unclear risk of bias for selection: recruitment to this study favoured women with an uncomplicated ongoing pregnancy. The risk of performance bias was low.

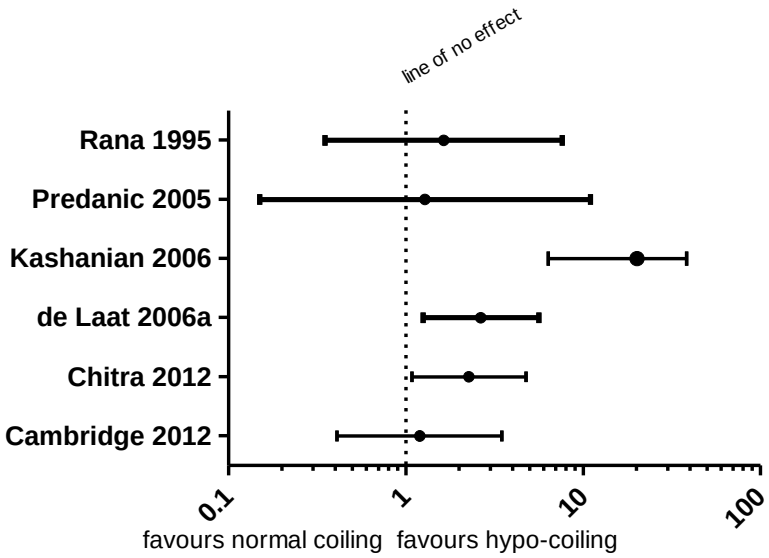
The results of analysis of heterogeneity (statistical and clinical) and risk of bias led to the conclusion that it presenting pooled quantitative data from the data currently available within the literature was not justified, nor calculation of odds ratios for the nominated outcomes. Forest plots are thus presented, in Figures 4.4 – 4.11, without pooled data icons. Analysis of findings in the forest plot format showed the data from the present study crossing the line of no effect for every outcome analysed. For a number of the other included studies – all of which reported findings as showing significant associations between variations in cord coiling and adverse outcomes – reanalysis of the published study data showed, for a number of outcomes, crossing of the line of no effect.



Odds ratio, M-H, fixed, 95% CI

Heterogeneity: Chi ^{2} =6.68, df=4 (P=0.14)

Figure 4.4. Forest plot of studies of hypo-coiled umbilical cords reporting small for gestational age infants as an outcome measure.



Odds ratio, M-H, fixed, 95% CI

Heterogeneity: Chi ^{2} = 17.78, df=5 (P=0.003)

Figure 4.5. Forest plot of studies of hypo-coiled umbilical cords reporting Apgar score <7 as an outcome measure.

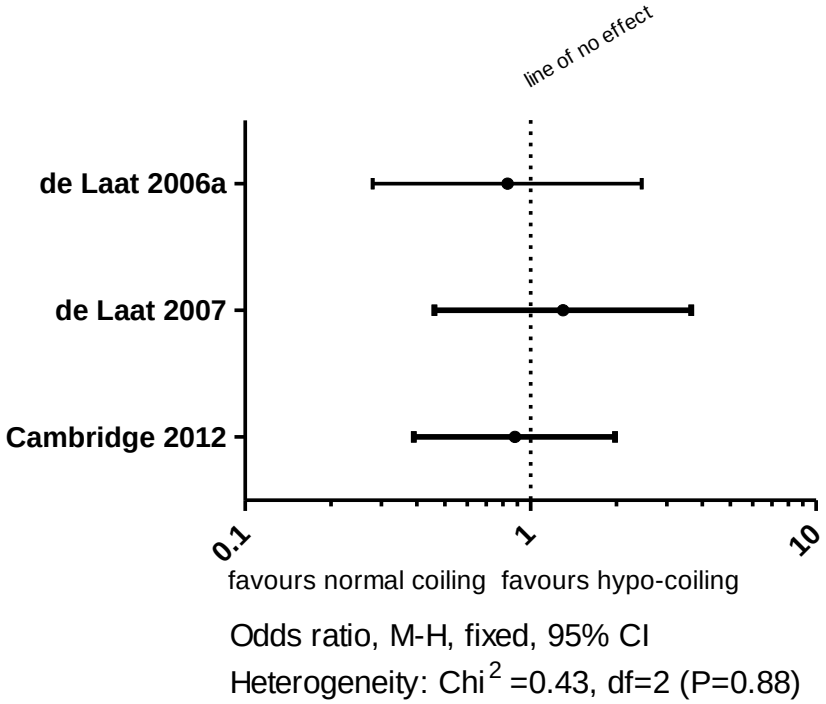


Figure 4.6. Forest plot of studies of hypo-coiled umbilical cords reporting neonatal acidosis as an outcome measure.

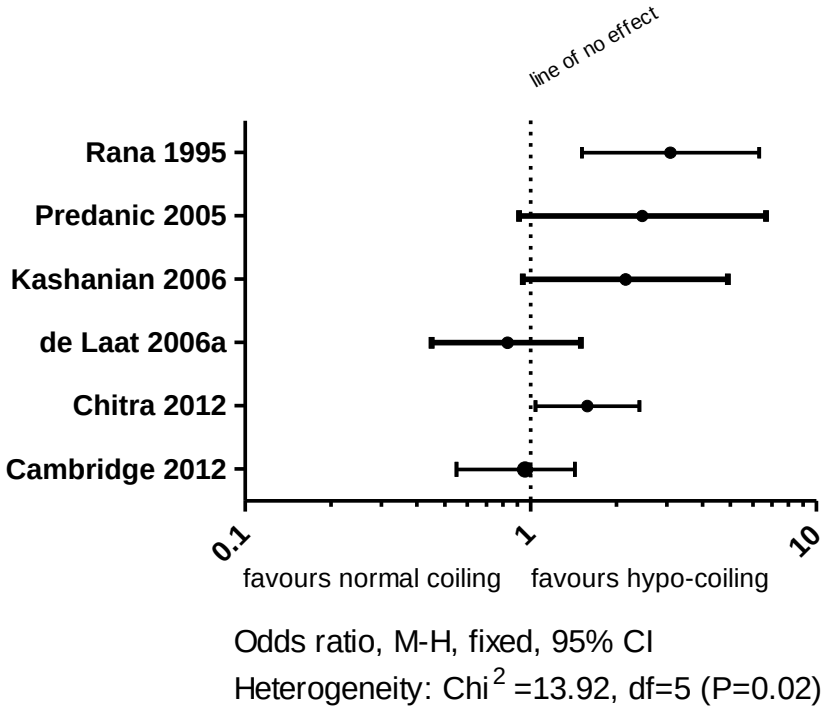
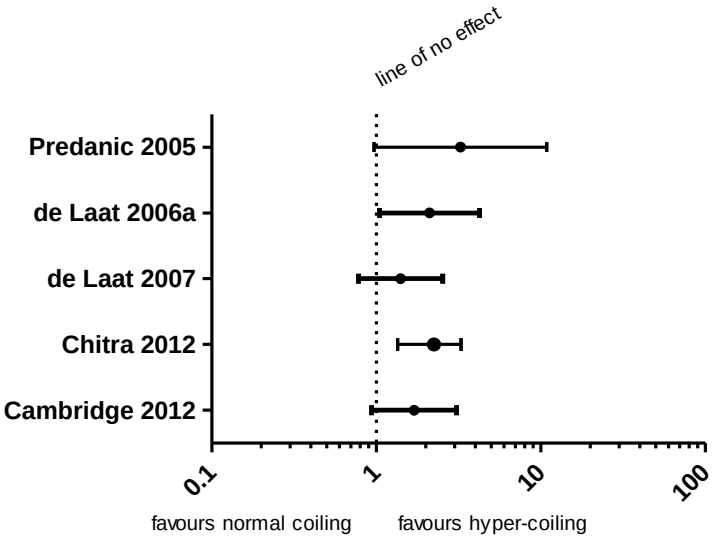


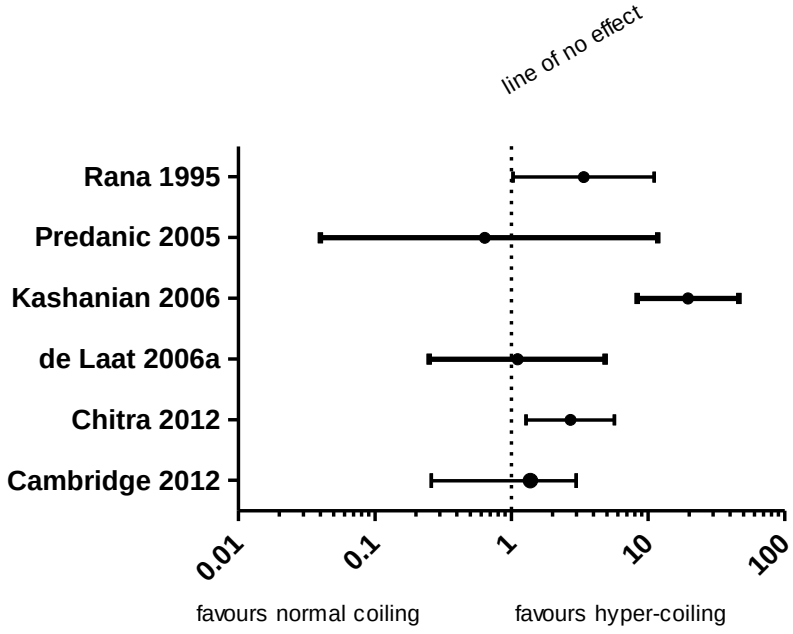
Figure 4.7. Forest plot of studies of hypo-coiled umbilical cords reporting interventional delivery as an outcome measure.



Odds ratio, M-H, fixed, 95% CI

Heterogeneity: Chi^2 = 2.17, df=4 (P=0.70)

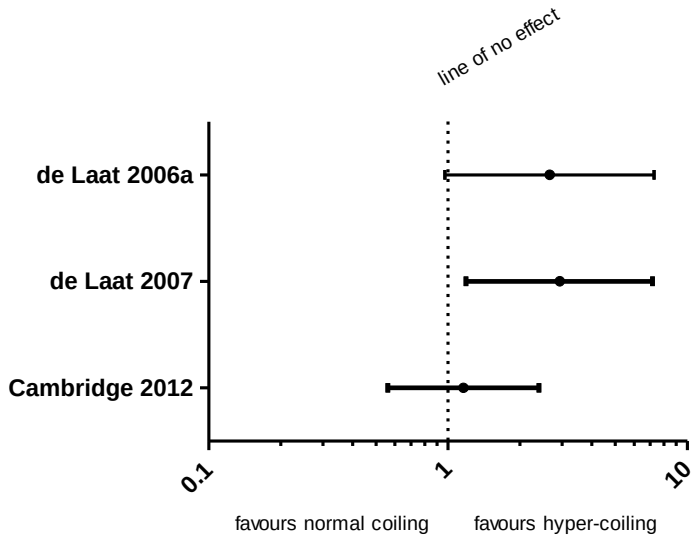
Figure 4.8. Forest plot of studies of hyper-coiled umbilical cords reporting small for gestational age infants as an outcome measure.



Odds ratio, M-H, fixed, 95% CI

Heterogeneity: Chi^2 = 24.71, df=5 (P=0.0002)

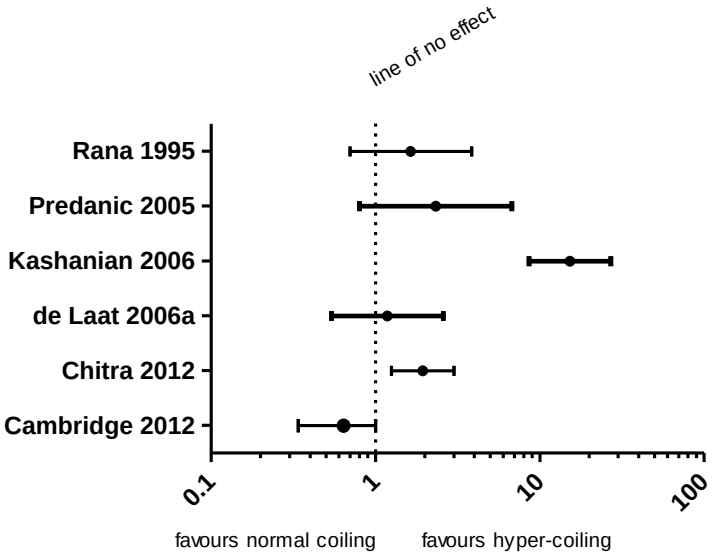
Figure 4.9. Forest plot of studies of hyper-coiled umbilical cords reporting Apgar score <7 as an outcome measure.



Odds ratio, M-H, fixed, 95% CI

Heterogeneity: $Chi^2 = 3.13$, df=2 (P=0.21)

Figure 4.10. Forest plot of studies of hyper-coiled umbilical cords reporting neonatal acidosis as an outcome measure.



Odds ratio, M-H, fixed, 95% CI

Heterogeneity: $Chi^2 = 71.21$, df=5 (P<0.00001)

Figure 4.11. Forest plot of studies of hyper-coiled umbilical cords reporting interventional delivery as an outcome measure.

4.5 DISCUSSION

No statistically significant association between hypo-coiled or hyper-coiled umbilical cords and a number of adverse neonatal outcomes has been demonstrated in this study. For hyper-coiled cords compared to normally coiled cords, a p value of 0.04 was found for two groups, namely interventional delivery and birth weight less than 10^{th} centile. In a study of this size, these findings should be viewed as non-significant. No other p values <0.05 were demonstrated.

The results of the Cambridge cohort are strikingly at variance with a number of studies previously reported within the literature (154, 155, 166, 170, 171, 173, 179, 180), despite cord coiling indices determined for the our hypo-coiled and hyper-coiled groups falling into ranges similar to those published for other populations (154, 155).

Consideration of the underlying reasons for this difference is an important question, particularly as cord coiling <10^{th} centile or > 90^{th} centile may be offered by placental pathologists as an explanation for adverse perinatal outcome and obstetricians are encouraged to consider how they might best determine and interpret the umbilical coiling index by ultrasound examination during pregnancy (168, 180, 183).

As broad a range of clinical outcomes as possible was included, by noting both absolute values (low birth weight, fetal acidosis and Apgar score <7) and clinical parameters which would indicate that the medical teams caring for the mother and infant had identified clinical factors which gave cause for concern.

Assessment of the Apgar score at delivery continues to be extremely valuable in predicting neonatal outcome, with scores ≥ 7 recognised as conferring a markedly reduced risk of neonatal death. The interpretation of neonatal pH, although a very commonly used measure of neonatal well-being, particularly immediately after birth, is complex (148). Many research studies rely on a pH ≤ 7.0 as a cut-off for identifying cases of severe acidosis. In our cohort, we raised the level to pH ≤ 7.2, a level used to identify cases of milder acidosis, to avoid excluding less severe but potentially significant acidosis

associated with variations in the cord coiling index. Birthweight < 10th centile was defined by standard United Kingdom 4 - in - 1 growth charts(184).

A decision to deliver a woman by interventional delivery is taken by the clinical obstetric team for a range of reasons: some maternal and some fetal. The decision thus relies on a broader assessment of intra-partum events than fetal tococardiography alone. It has been used as such in a number of previous studies of the clinical associations of umbilical cord coiling out with the perceived range of normal. Admission to the neonatal special care unit is again a variable which depends on clinical decision-making but reflects sufficient impairment in one or more neonatal organ systems as to require specialist care. This study benefits from consistent clinical guidelines applied within one centre and these clinical outcomes were thus included as potentially useful.

The selected parameters in this study are therefore sufficiently wide ranging and robust as to have captured infants who had any degree of compromise present at birth. Why, then, do these results appear to be different when compared to a number of previously published studies?

In order to at least partially address this question, a systematic review of previously published cord coiling studies was undertaken, with inclusion criteria as broad as the format could accommodate.

A number of the studies included in the systematic review have previously reporting significant associations between hypo-coiled and hyper-coiled umbilical cords. Extraction of the data and re-analysis in the forest plot format did not, for a number of outcomes, show any evidence of a significant effect.

Overall, considerable statistical and clinical heterogeneity was found, together with high risks of bias when included studies were considered in the setting of the Cochrane review model. The systematic review model did not, therefore, support definite conclusions in favour of abnormal cord coiling as an abnormality associated with the perinatal outcomes studied.

The strength of the Cambridge cohort study lies in the unselected nature and size of the cohort with selection of a small number of clinical outcomes of real relevance to the

wellbeing of the neonate: some of the variability reported amongst the previously published studies may be associated with the large number of outcomes studied. Further studies carried out on unselected prospective cohorts with clearly defined, important clinical outcomes, are likely to clarify the mixed messages now in existence relating to this area of placental study.

4.6 CONCLUSIONS

- 1082 cases with umbilical cords > 15cm were identified in the cohort of 1119.
- The cord coiling indices calculated for these 1082 cases in this study are similar to those published for other populations
- Five specific neonatal outcomes in infants were recorded:
    - Interventional delivery (252/1082)
    - Birthweight < 10^{th} centiles (100/1082)
    - Apgar score < 7 at one minute of age (34/1082)
    - Neonatal acidosis (pH < 7.2) (79/1082)
    - Admission to the neonatal intensive care unit (66/1082)
- No statistically significant associations relating adverse outcomes to cords below the 10^{th} centile were found.
- No statistically significant associations relating adverse outcomes to cords above the 90^{th} centile were found.
- Systematic review of the literature identified six studies fulfilling the criteria for inclusion
- The data from a number of included studies was shown, in the forest plot format, to cross the line of no effect.
- Heterogeneity analysis (both statistical and clinical), together with risk of bias assessment, indicated that generating pooled quantitative data with generation of odds ratios could not be justified.
- There is no evidence, either from the data generated in this study or from a systematic review, to support the conclusion that variations in umbilical cord coiling indices are associated with adverse neonatal outcomes.

CHAPTER 5: CLINICAL CORRELATION OF MACROSCOPIC AND MICROSCOPIC PLACENTAL INFARCTION IN AN UNSELECTED POPULATION, WITH IMMUNHISTOCHEMICAL ANALYSIS

Summary:

- Placental perfusion
- Assessment of fetal and placental growth: raw data and z scores
- Variations in cord insertion site and placental shape
- Identification of placental infarcts: macroscopic and microscopic
- Relationships between placental infarcts, maternal hypertension, fetal/placental growth and variations in cord insertion site or placental shape
- Pilot immunohistochemical studies

5.1 INTRODUCTION

The underlying event in infarction of biological tissues is, in most organ systems, attributable to occlusion of the local arterial blood supply. This is self-evident for very common lesions in other tissues – typically infarction of the myocardium secondary to coronary artery occlusion – but is more complex for the human placenta, which receives a dual blood supply from the left and right uterine arteries. The chorionic villi are vascularised by branching of fetal vessels, but are also in direct contact with the maternal blood in the intervillous space. There is little evidence in the more recent literature for the view that the fetal vasculature is primarily compromised in the pathogenesis of placental infarction, and studies of placental perfusion suggest that, although the micro-architecture of the placenta may be suggestive of a freely communicating intervillous lake of maternal blood, functional studies are much more in keeping with maternal spiral arteries functioning as end arteries (3). Presumed occlusive events within these end arteries, either within the more superficial maternal decidua which separates from the placental bed at parturition or (probably more commonly) within the deeper maternal spiral arteries, results in placental parenchymal infarction (34, 83, 122). A schematic depiction of human placental micro-circulation is given in Figure 5.1 (after L'Herminé-Coulomb) (185).

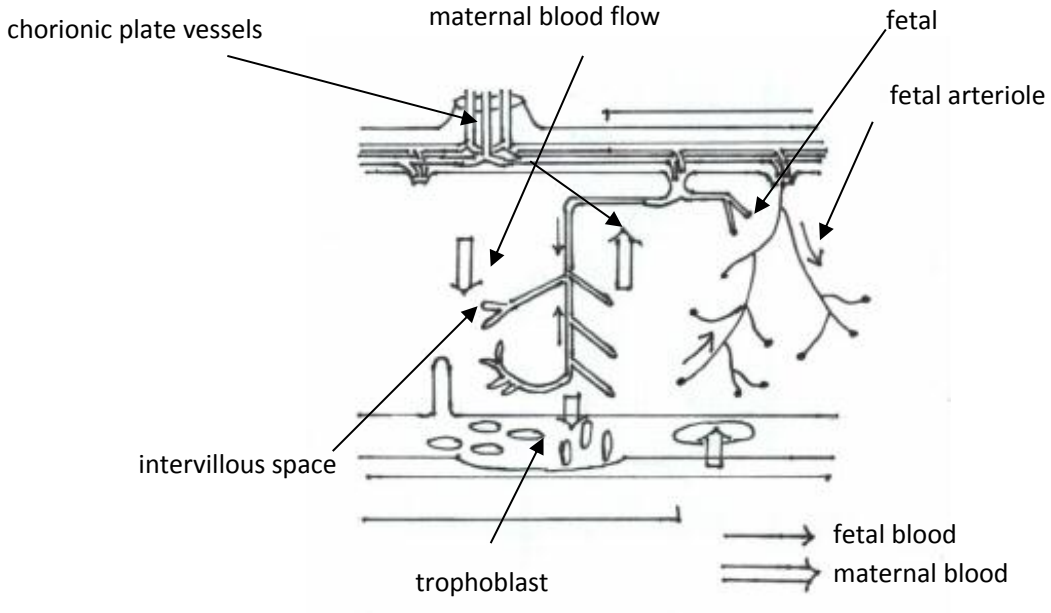


Figure 5.1: human placental micro-circulation

Second and third trimester growth and later development of the placenta (and fetus) are dependent on appropriate transformation of maternal uterine arteries, mediated by

trophoblast invasion of these vessels. Deficiencies in trophoblast invasion are linked to placental oxidative stress, placental infarction and fetal growth restriction (186, 187). This pathway has thus received considerable attention in the setting of preterm, small for gestational age infants (90). The clinical outcomes and thus significance of term placental infarction are less certain: placental infarction is considered in multiple clinical studies to be associated with small for gestational age infants at term (20, 27, 29, 34, 131, 161, 188, 189), but few unselected cohort studies of outcomes in placental infarction at term have previously been reported (20, 34).

The relative importance of recording and analysing birth weight alone, placental weight alone or a combined birth:placental weight ratio remains controversial: fetal and placental weight have been reported to be strongly correlated (190, 191) and a number of studies report that variations in birth:placental weight ratios are associated with adverse outcomes (188, 192, 193). This analysis has, however, been criticised in that a "normal" birth:placental weight is calculated when both birth and placental weight are normal, low or high (194), and z scores have been advocated as a more robust form of analysis in the investigation of fetal and placental growth (138, 194).

The specific mechanisms of disease which cause arterial lesions in placental infarction are not well understood. A proportion of placental infarcts are consistently associated with specific maternal pathology: hypertensive disorders (30, 44, 195-197), factor V Leiden deficiency (198), antiphospholid syndromes (199) and other maternal connective tissue disorders (200). The remainder of recognised placental infarcts are idiopathic.

Macroscopic variations in umbilical cord insertion (201, 202) and placental disc shape (203) have been advanced as an explanation for placental "insufficiency" and a wide range of abnormal pathways in cell biology have been investigated to account for apparent placental hypoxia (186, 204-207), although the majority of studies in this field are predominantly directed towards investigating growth in the context of pre-eclampsia.

In this study, the relationships between placental infarction and both fetal and placental growth are investigated in some detail, with growth analysed both as raw data and as z scores. Quantification of infarcts against fetal and placental growth is also reported.

Possible pathways accounting for placental infarction are investigated at multiple levels: clinical, macroscopic and microscopic. The relationship between maternal hypertensive disorders and placental infarction in this cohort is examined. At the macroscopic level, variations in umbilical cord insertion site and placental eccentricity in the presence or absence of infarcts are investigated. Finally, the analysis of pilot immunohistochemical studies of possible cell biology pathways implicated in the pathogenesis of placental infarction is reported.

5.2 METHODS

Macroscopic and microscopic analysis of placentas, photography and neonatal data collection were carried out as described in Chapter 2, section 2.2.3.

ImageJ software was used to calculate both the percentage infarction of the cut surface of placentas with macroscopically recognised infarction, and to calculate the area of villi in each case analysed immunohistochemically, again as described in Chapter 2, sections 2.2.5 and 2.2.11. For antigens identifying individual cells (eg CD3), total positive cell counts were recorded. For cell surface expression of antigen, each positive villus was recorded.

Calculation of indices expressing variations in cord insertion and placental disc eccentricity have previously been reported (24): briefly, the cord centrality index is expressed as a value between 0 and 1. The smaller the cord centrality index, the closer to the centre lies the cord insertion site and, conversely, the larger the cord centrality index, the further away from the centre it lies. Eccentricity index is expressed on the basis of a mathematical formula which expresses a perfect circle as a value of 0 with a maximally elliptical shape as a value of 1.

5.3 STATISTICAL ANALYSIS

Noting the discussion above relating to the relative importance of birth weight, placental weight and birth:placental weight ratio, multiple analyses were undertaken, The initial analysis performed was that an uncorrected comparison of infarct to non-infarct cases by birth weight, placental weight and birth:placental weight ratio. Subsequently, calculation of z scores, which assess the deviation from the mean for each value analysed, was carried out on a gestational age (completed gestational week) specific basis.

Statistical testing was undertaken with OpenEpi (178) and GraphPad (146) software, with generation of t test and Spearman rank correlation co-efficient p values.

5.4 RESULTS

All cases of placental infarction occurred in cases of 37-42 weeks gestation, and the control group was thus restricted to cases from the same gestational age group. Infarcts were identified in a total of 55 (5.1%) cases – 43 (4.0%) cases had a macroscopically identifiable infarct (subsequently confirmed histologically), with a further 12 (1.1%) of cases noted to have parenchymal infarction on microscopic examination of cases with no observed macroscopic abnormalities. For the 43 cases with a macroscopically recognised infarct, further analysis of the number of recognisable infarcts and the percentage of recognisably infarcted placental parenchyma was undertaken. These data are presented in Table 5.2. Twenty nine macroscopically recognised cases of infarction included only one or two infarcts, with fourteen cases including three or more: the mean number of infarcts/case was 2.44 (SD 2.71) and the mean percentage infarction/case was 1.36% (SD 2.09).

As noted, all 55 cases of infarction fell into the gestational age range 37-42 weeks. Only one case was >41 weeks and so infarct cases were stratified into 37, 38, 39, 40 and 41+ completed gestational weeks. This gestational age range was applied to the control (non-infarct) group which, with the exclusion of cases of gestation < 37 weeks and > 42 weeks, numbered 1025. There was no statistical significance between the number of cases for each completed gestational week for infarcts compared to non-infarcts (see Table 5.1 and Figure 5.2).

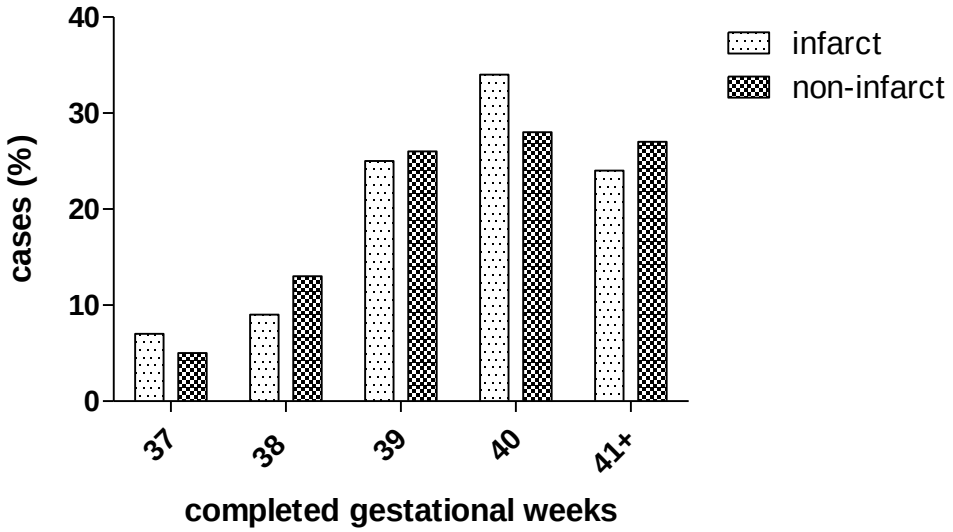


Figure 5.2: infarcted placentas vs non-infarcted placentas by gestational week

	Infarct (%) n=55	**Non-infarct (%) n=1025**	**p value**
37 weeks	4 (7)	55 (5)	0.71
38 weeks	5 (9)	136 (13)	0.51
39 weeks	15 (25)	271 (26)	1.0
40 weeks	19 (34)	287 (28)	0.36
41 + weeks	13 (24)	276 (27)	0.72

Table 5.1: Infarcted placentas vs non-infarcted placentas by gestational week

NO. OF INFARCTS	% INFARCT	CUT SURFACE AREA (cm2)	INFARCT SURFACE AREA (cm2)
1	0.74	270	2.0
1	0.37	207	0.76
1	0.27	175	0.47
1	0.71	240	1.7
1	0.21	238	0.5
1	0.47	236	1.1
1	0.31	180	0.56
1	0.16	209	0.34
1	0.21	221	0.47
1	0.60	218	1.3
1	0.48	158	0.76
1	0.65	262	1.7
1	0.88	159	1.4
1	0.47	224	1.0
1	0.30	265	0.8
1	0.64	234	1.5
1	0.74	162	1.2
1	0.08	130	0.1
1	0.21	191	0.4
1	0.79	266	2.1
1	0.79	189	1.5
1	0.40	170	0.68
2	1.4	189	2.6
2	1.6	165	2.7
2	2.7	265	7.1
2	1.3	176	2.3
2	0.35	173	0.6
2	2.2	169	3.7
2	0.74	270	2.0
3	0.59	158	0.93
3	5.0	242	12
3	0.90	265	2.4
3	0.85	223	1.9
3	0.80	201	1.6
3	1.3	202	2.6
4	1.1	160	1.8
4	3.4	160	5.4
4	3.2	169	5.4
4	0.75	173	1.3
6	3.2	278	9.0
6	2.1	165	3.4
6	1.5	177	2.6
17	13	138	18

Table 5.2: Analysis of macroscopically recognisable placental infarcts

Birthweight and placental weight analyses

Calculation of p values for birthweight, placental weight and birth:placental weight ratio for infarcted and non-infarcted placentas showed statistically significant differences for birthweight but not for placental weight or birth:placental weight. These findings are presented graphically in Figures 5.3-5.5.

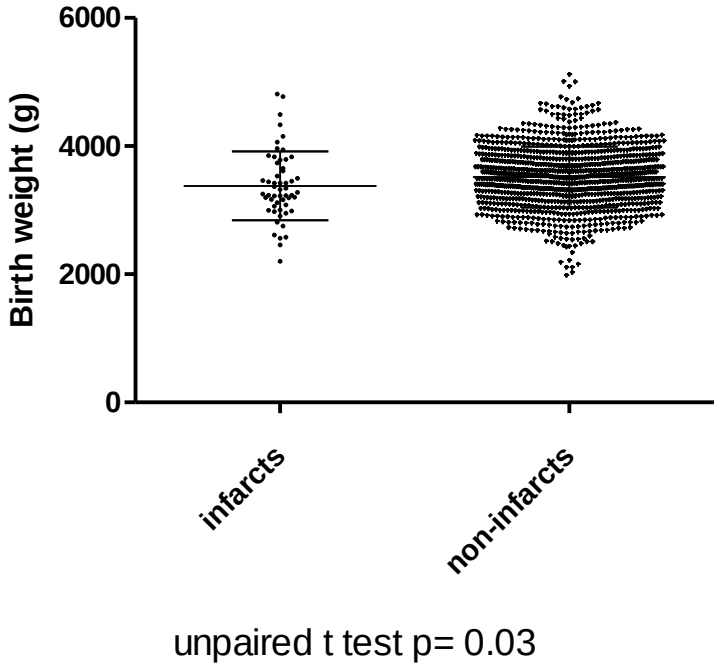
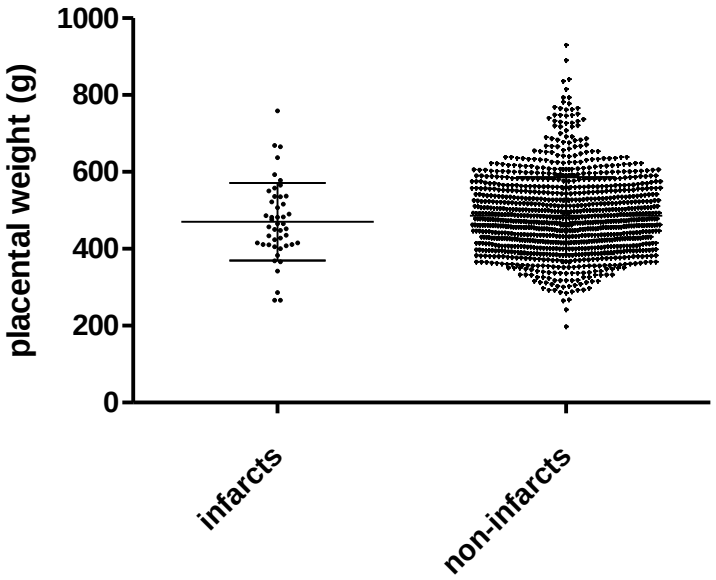
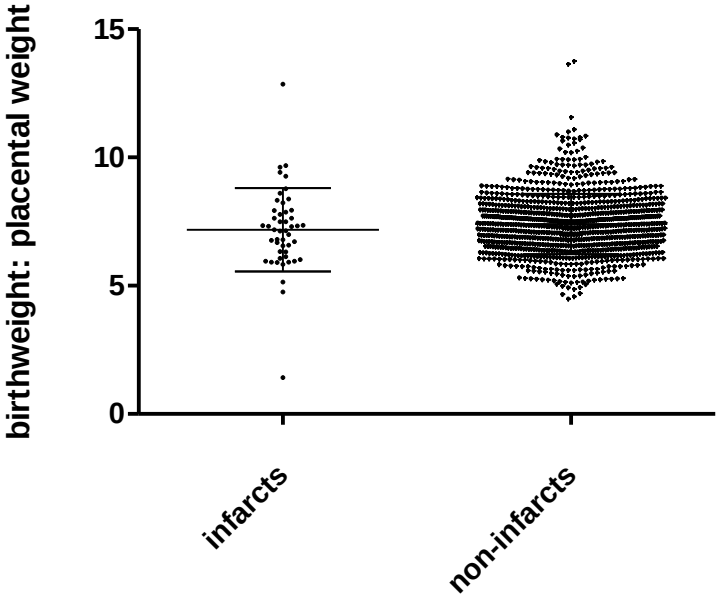


Figure 5.3 Birthweight, infarcts vs controls
(uncorrected for gestational age, 37 - 42 weeks)



unpaired t test p= 0.29

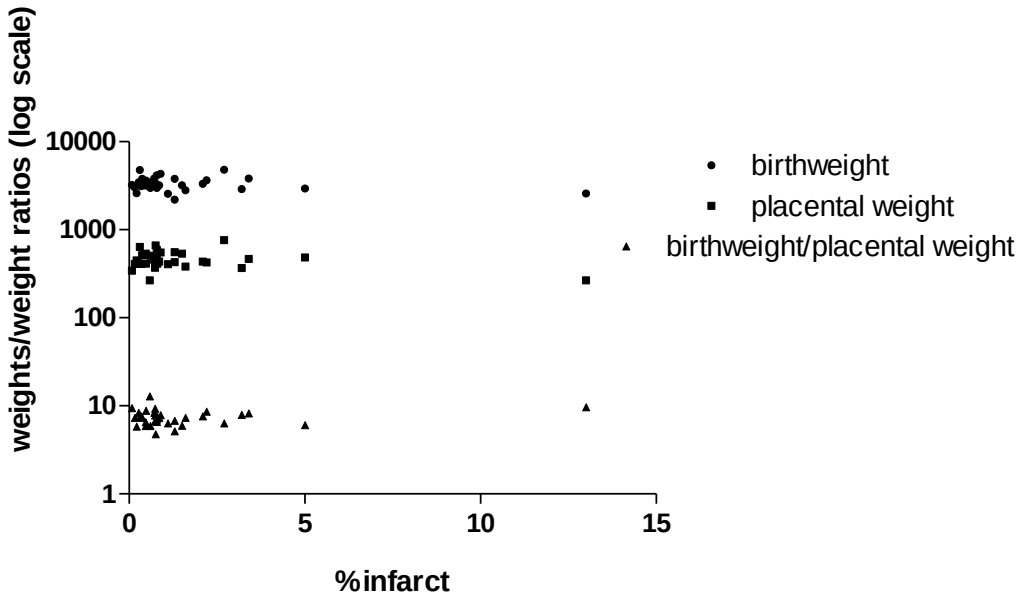
Figure 5.4. Placental weight, infarcts vs controls (uncorrected for gestational age, 37 - 42 weeks)



unpaired t test p= 0.21

Figure 5.5. Birthweight:placental weight ratio, infarcts vs controls
(uncorrected for gestational age, 37 -42 weeks)

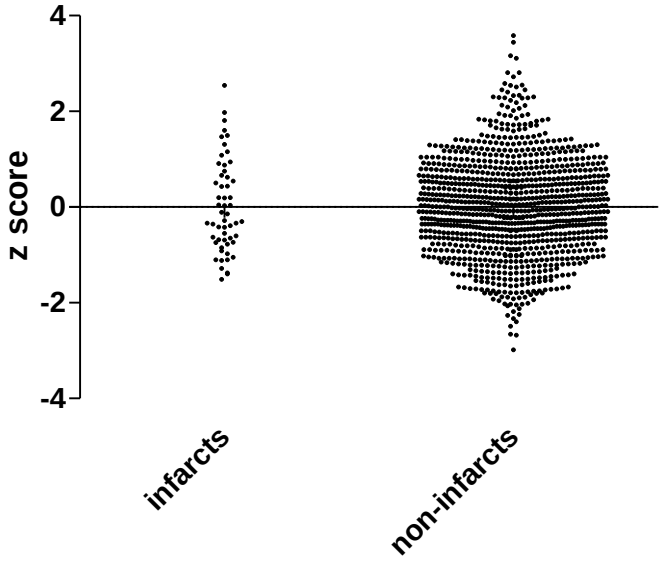
The relationships between percentage infarction and birth weight, placental weight and birthweight:placental weight were also examined. There was no statistical correlation between percentage of placental infarction and any of these variables in this study. These data are presented in Figure 5.6.



	Birthweight vs % infarction	Placental weight vs % infarction	Birthweight:placental weight vs % infarction
Spearman's rank correlation coefficient	-0.12	0.04	-0.03
p value	0.47	0.81	0.86

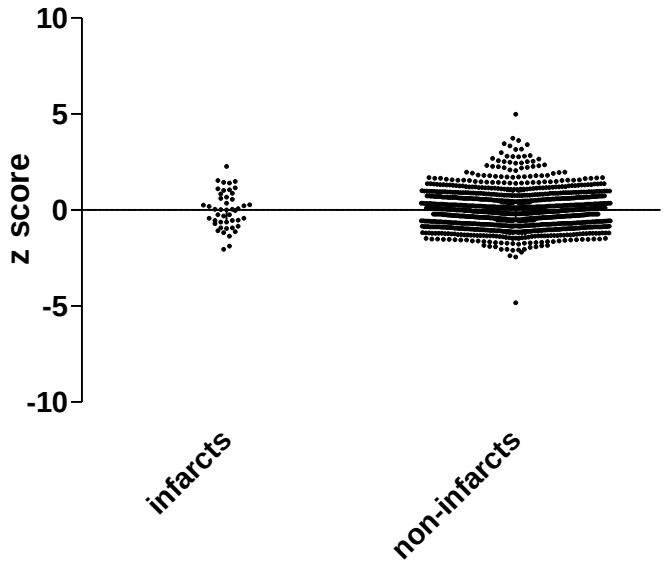
Figure 5.6. Scatterplot of birthweight, placental weight and birthweight:placental weight ratio vs % placental infarction

The significant p value for birthweight when comparing infarcts and non-infarcts noted, the gestation specific z scores were compared for birth weight, placental weight and birthweight:placental weight. These showed no significant differences for infarcts compared with non-infarcts for these three growth parameters. These findings are presented graphically in Figures 5.7 -5.9. Percentage infarction against z score birthweight is shown in Figure 5.10. This analysis was again statistically non-significant.



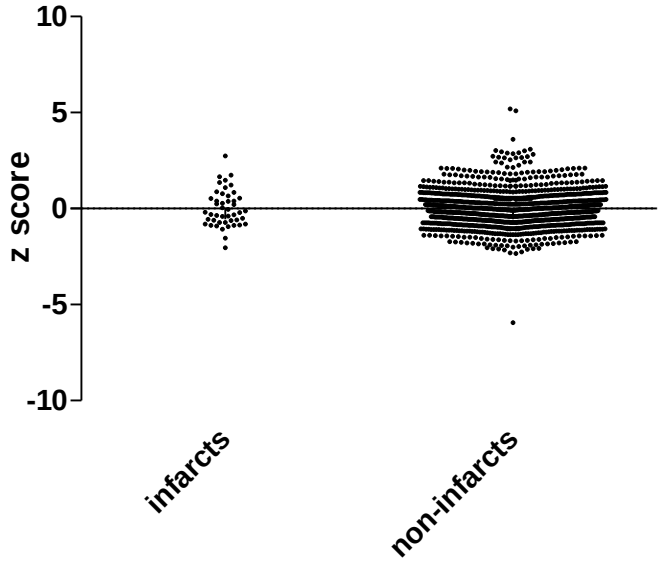
unpaired t test: p=0.72

Figure 5.7. z scores (birth weight), infarcts vs controls



unpaired t test: p=0.82

Figure 5.8. z scores (placental weight), infarcts vs controls



unpaired t test: p=0.95

Figure 5.9. z scores (birthweight:placental weight), infarcts vs controls

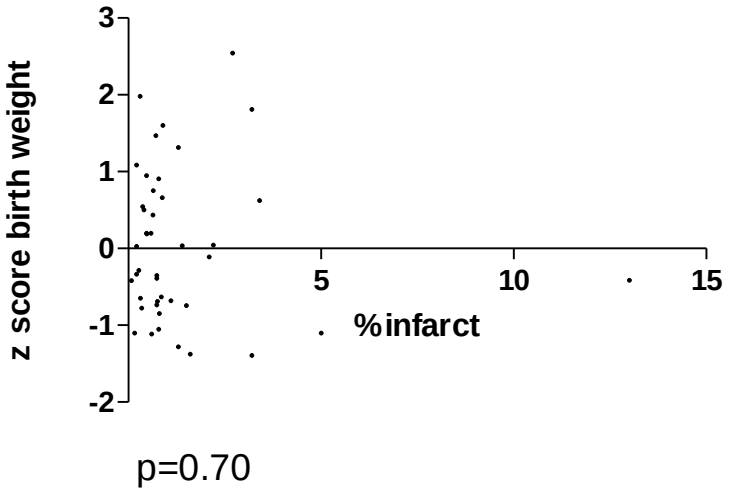


Figure 5.10. z score birth weight vs placental infarction (%)

Maternal hypertensive disorders and placental infarction

Three subjects, out of the 55 found to have identifiable placental infarction, had a pregnancy-related hypertensive disorder. Given the low incidence, no further subdivision of hypertensive disorders was undertaken. Of the 1025 controls, 48 had a pregnancy-related hypertensive disorder.

As shown in Table 5.3, there was no significant difference between the incidence of hypertensive disorders of pregnancy in the infarct vs control groups.

	Maternal hypertension	**Maternal normotension**	**Totals**
Infarcts	3	52	55
Non-infarcts	48	977	1025
Totals	51	1029	1080

p = 0.75

Table 5.3. Two by two table, maternal hypertensive disorders vs placental infarction

Cord centrality/placental eccentricity and placental infarction

Correlation of the cord centrality and eccentricity indices calculated elsewhere (24) with infarcts versus non-infarcts showed no significant correlation between cord centrality or placental eccentricity. These findings are presented in Figures 5.11 and 5.12.

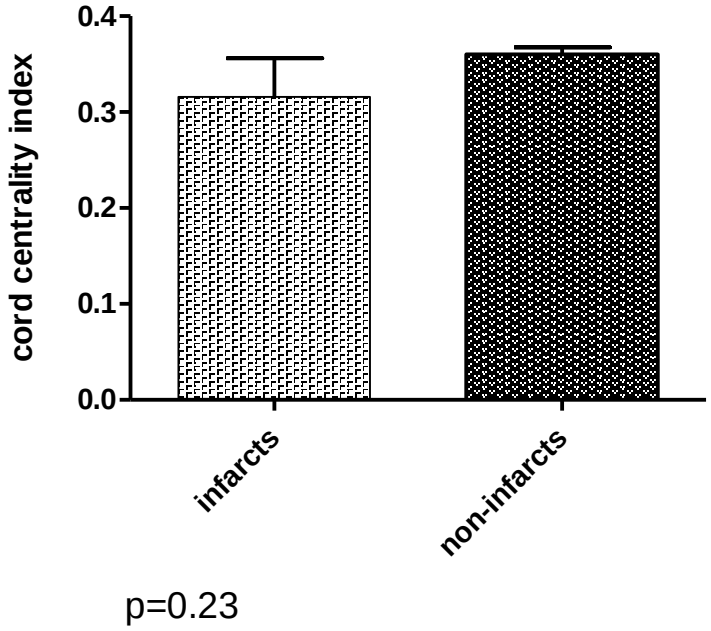


Figure 5.11. Cord centrality index, infarcts vs controls

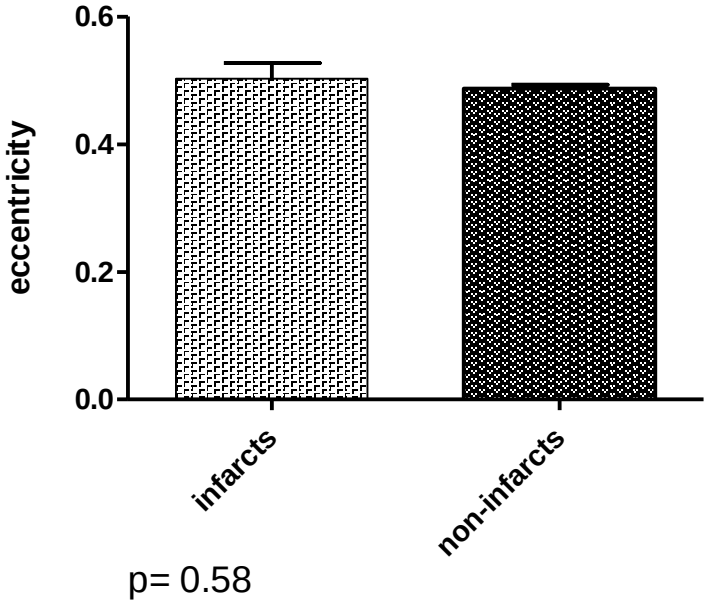


Figure 5.12. Placental eccentricity index, infarcts vs controls

Immunohistochemical studies

Immunohistochemical staining and analysis was carried out on two sets of micro-arrays, placental tissue just adjacent to a focus of infarction and placental control tissue. Each microarray comprised 20 cases, with preparation and immunohistochemical staining as described in 2.2.9 and 2.2.10. The target cell type/antigen is given in Table 5.4. The results of scoring each micro-array are given in Figure 5.14.

Primary antibody	Cell type/antigen
CD3	T cell
CD20	B cell
HLADR	cell surface class ii major histocompatibility complex
CD68	macrophage/monocyte series
C4d	complement activation
cleaved caspase 3 (Cl-Cas3)	apoptosis marker
ICAM (CD54)	leukocyte/endothelial transmembrane adhesion molecule
P selectin (CD62P)	activated endothelial/activated platelet transmembrane adhesion molecule
Fox P3	T regulatory cell (TREG) phenotype

Table 5.4. Immunohistochemical panel: target cell type/antigen

Given that these were pilot studies, no statistical analysis was performed on These data. Initial results suggested that C4d and cleaved caspase 3 may be of further interest in definitive immunohistochemical studies. C4d decorated the syncytiotrophoblast of chorionic villi adjacent to infarction, while cleaved caspase 3 had a granular cytoplasmic staining pattern in villous stromal cells.

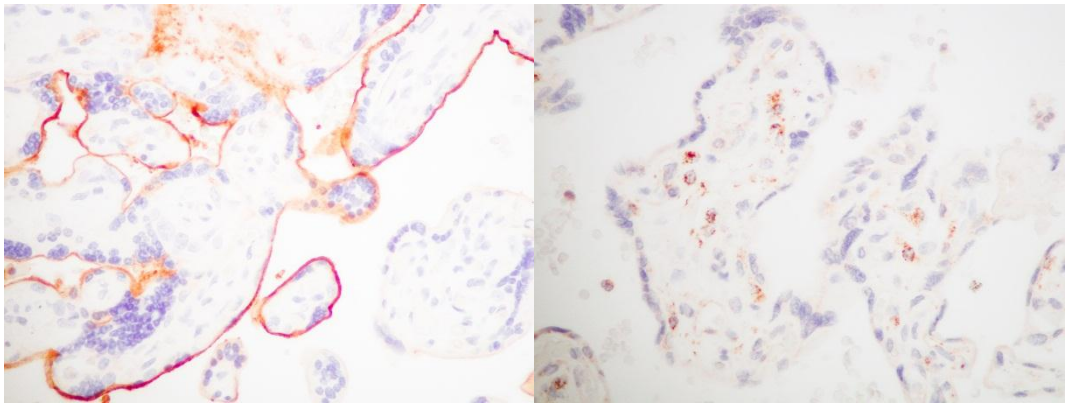
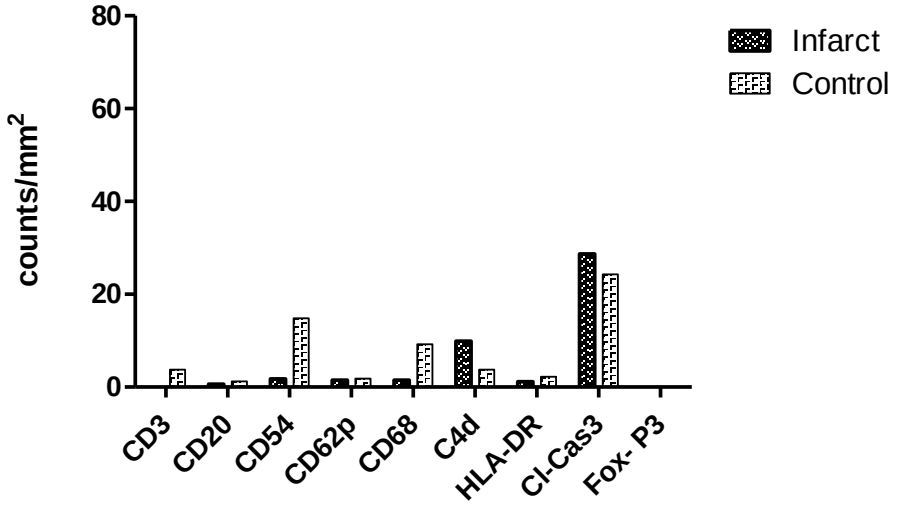


Figure 5.13 Immunohistochemical expression of C4d (left) and cleaved caspase 3 (right) adjacent to a focus of placental infarction (x 10 objective).



	Infarct			Control		
	Mean count	**SD**	**n**	**Mean count**	**SD**	**n**
CD3	0.00	0.00	20	3.76	2.93	20
CD20	0.64	0.68	20	1.20	1.36	20
CD54	1.75	6.92	20	14.80	34.20	20
CD62p	1.48	2.51	20	1.86	2.49	20
CD68	1.51	2.62	20	9.25	15.10	20
C4d	9.90	14.10	20	3.76	13.80	20
HLA-DR	1.18	1.25	20	2.22	3.18	20
Cl-Cas3	28.70	41.90	20	24.30	34.60	20
Fox- P3	0.00	0.00	20	0.00	0.00	20

Figure 5.14. Immunohistochemical analysis, placental infarction vs control

5.5 DISCUSSION

Placental infarcts are macroscopically and microscopically recognisable lesions where the placental parenchyma has undergone necrosis – with, as noted above, most evidence supporting occlusion of maternal end arteries as the primary event. Most studies reporting outcomes in placental infarction focus on fetal growth/birth weight as the outcome endpoint, and this model was adopted in the present study.

Very few studies exist which report on infarction in unselected cohorts delivering at term. The majority reporting a definite association between growth restriction and placental infarction are, as discussed in Chapter 3, case control studies, with an estimate of the true incidence within a population only available from an analysis of an individual study's control group.

This study reports a significant association between birthweight and infarction (in comparison to the non-infarcted cases in this cohort) only on analysis of the raw data. When standardised for gestational age, by calculation of the z score, this effect disappeared. The z score data are to be preferred in this context, as each value is expressed as a variation from the mean for each gestational week. Associations between the percentage infarction of each placenta in which macroscopic infarcts were identified and birthweight, placental weight and birthweight:placental weight were also explored: no association was shown in this study. There was no association between maternal hypertensive disorders and placental infarction in this study.

Previous studies have reported only on raw data in a case control model, and two of the largest (20, 34) date from 2002 and 1992, respectively. There may thus be multiple factors which account for the differences between this study and those previously published.

First, the case control model introduces a risk of bias. It is much more difficult to avoid selection bias in the non-random selection of cases: however conscientiously the researcher attempts to exclude bias, the case selection may be stratified for both abnormal and control groups. There is also, depending on the study design, the possibility that the researchers are not blinded when assessing the macroscopic and microscopic appearances of the placentas.

Secondly, obstetric management may have biased the findings in this study against the detection of all placental infarcts in the baseline population – intervention, by delivery of the infant on a semi-elective or emergency basis, may have resulted in diminished opportunities to recruit some cases of placental infarction, given that the design of the present study resulted in a bias towards recruitment of uncomplicated pregnancies delivering at term.

Thirdly, the populations examined in the small number of studies reporting on term placental infarction may be quite variable, both in terms of ethnic origin and smoking (20) and maternal pre-conceptual body mass index (34). The Cambridge population, as noted in Chapter 3, was a predominantly low-risk Caucasian population.

Nevertheless, the data that are available from this study support the conclusion that the incidence of maternal hypertension, birth weight, placental weight and birth:placental weight ratio, in pregnancies delivering between 37 and 42 weeks, show no evidence of variation between those cases showing placental infarction and those without.

The cord centrality index and placental eccentricity index were examined for association with placental infarction. As demonstrated, there was no apparent association between the these variables and placental infarction. This study thus provides no evidence that placental infarction may be the underlying lesion in those clinical studies reporting placental "insufficiency", or reduced efficiency, in the context of variable cord insertion and placental shape (16, 25, 201, 202).

A further objective of this study was to evaluate possible immunohistochemical markers in the elucidation of pathways of placental infarction. A pilot study of immunohistochemical staining, with a range of markers, carried out on 20 cases of placental parenchyma adjacent to an infarct and 20 controls, identified C4d and cleaved caspase 3 as markers of interest for more definitive studies.

The finding that C4d shows, in these pilot studies, an increase in staining in viable chorionic villi adjacent to areas of infarction is of interest given recent studies reporting activation of complement in placentas delivered by women diagnosed with hypertensive disorders of pregnancy, antiphospholipid syndrome or systemic lupus erythematosus (208, 209). These studies describe statistically significant associations between C4d and disease

compared with normal controls, and conclude that activation is via the antibody mediated classical pathway. In the present study, however, the presence of C4d expression adjacent to foci of placental infarction in low-risk pregnancies with no statistically significant adverse outcome raises the possibility that the presence of C4d is possibly a non-specific marker for complement activation via the lectin pathway, triggered by tissue damage. In this context, the observation that apoptotic placental tissue is observed to activate complement systems (43, 81, 82) is highly relevant: the suggestion that there may be a threshold effect to clinically significant apoptosis and complement activation (82) is attractive and merits further evaluation in the context of low-risk, normal outcomes pregnancies with identifiable placental infarcts.

Cleaved caspase 3 was also found to have increased expression in chorionic villi adjacent to placental infarcts compared to normal controls. In many ways, this finding has parallels with the findings for C4d expression in this study. Activation of placental apoptosis is reported to be associated with pre-eclampsia (210) and the caspases are certainly a family known to be highly involved in the intracellular apoptotic pathway (92). Again, investigation of the pattern of expression of a more extended set of apoptosis markers in low-risk, normal outcome pregnancies with identifiable placental infarcts is likely to provide clearer insights into the relationships between apoptosis and adverse outcome.

5.6 CONCLUSIONS

- 55 cases of placental infarction identified
- No statistically significant difference in incidence of placental infarction by gestational week (37-42) compared with controls
- No statistically significant difference in placental weight and birth:placental weight in analysis of raw data or z scores
- Marginally statistically significant difference in birth weight on analysis of raw data, not sustained on z score analysis
- No statistically significant correlation between percentage placental infarction and birth weight, placental weight or birthweight:placental weight
- No statistically significant difference in incidence of infarcts in maternal hypertensive disorders compared to normotensive controls
- No statistically significant correlation between infarcts and cord centrality index or placental eccentricity
- Pilot immunohistochemical studies identify increased C4d and cleaved caspase 3 (markers of complement activation and apoptosis) expression in chorionic villi adjacent to placental infarction compared to controls

CHAPTER 6: CLINICAL CORRELATION OF ACUTE PLACENTAL HISTOLOGIC INFLAMMATION PATTERNS IN AN UNSELECTED POPULATION

Summary:

- Cases showing any degree of acute inflammation identified
  - Cases classified as abnormal by current reporting protocols
  - Cases showing any degree of acute inflammation
- Five specific neonatal outcomes identified
  - Interventional delivery
  - Birthweight
  - Apgar score < 7 at one minute of age
  - Neonatal acidosis (pH < 7.2)
  - Admission to the neonatal intensive care unit
- Associations between acute inflammation and clinical outcomes examined
- Fetal inflammatory response syndrome reviewed

6.1 INTRODUCTION

Chorioamnionitis and funisitis are defined histologically as acute inflammatory responses in, respectively, the fetal membranes and umbilical cord. There is longstanding accumulated evidence that the observed acute inflammation is attributable to ascending genital tract infection. The presence of inflammation in cord and membranes is reported to have considerable implications for both early and late well-being in pre-term neonates, with multiple major studies reporting adverse outcomes in clinical and histological chorioamnionitis (74-76, 106, 211-214). Interestingly, conflicting evidence exists for some clinical outcomes in pre-term chorioamnionitis - for example, the lesion is reported to be both positively (106) and negatively (214) associated with pre-term intra-ventricular haemorrhage. The effects of chorioamnionitis on premature infant lung function also appear complex with apparently contradictory findings of decreased frequency of short - term respiratory distress syndrome but increased frequency of long-term chronic lung disease/bronchopulmonary dysplasia. (215). Regardless of the specific positive or negative effects of chorioamnionitis, the pathway by far the most commonly cited as inducing a physiological effect on the pre-term infant is the fetal inflammatory response syndrome, mediated by interleukin 6 and thought likely to be of considerable importance in the setting of a leaky, immature blood brain barrier (216-218).

For term deliveries, the significance of an acute histological inflammatory chorioamnionitis or funisitis is less clear. Publications describing clinical outcomes in term pregnancy are much less frequent, and predominantly explore associations between the development of chorioamnionitis and cerebral palsy (118). It has previously been reported, however, that there is no evidence of an association between relatively severe term neonatal acidosis and intra-uterine infection (219). A recent report raises the controversial hypothesis that term chorioamnionitis may not be infective in origin (158).

Assessment of the significance of acute inflammation in placentas delivered from term pregnancy is, however, an important question – not least because, as guidelines for routine clinicopathological assessment of the placenta accumulate, the onus on the individual reporting pathologist is to recognise all possible pathologic lesions, while disregarding histological patterns of no or limited diagnostic significance. Resolving these issues would also be of considerable interest to practising obstetricians and neonatologists in

determining which placentas to submit for histological examination and in understanding the significance of the resulting histopathology report.

This study provides a rare opportunity to consider the clinical outcomes of acute inflammatory patterns in varying compartments of placental microanatomy, in the specific clinical setting of low-risk pregnancies delivering around term.

6.2 METHODS

Detailed accounts of recruitment to the study, recording of clinical data, microscopic and microscopic placental examination are given in Chapter 2.

6.3 STATISTICAL ANALYSIS

Birth weight amongst subgroups was, as a continuous variable, analysed by one way ANOVA testing.

For clinical associations, which take the form of discrete variables, Fisher's exact and mid P values were calculated, together with odds ratios and 95% confidence intervals.

The statistical tests used are further discussed in Chapter 2.

6.4 RESULTS

As noted in Chapter 3, 188 cases out of the 1119 included in this study showed some degree of acute inflammation. Based on current clinical practice (3), 121 of these 188 would have been reported as potentially significant by a routine diagnostic histopathologist: the remaining 67 were cases showing changes which were judged to be, on the first round of reporting, minor infiltrates of polymorphs of no diagnostic significance.

The numbers of cases in each subgroup following a second round of light microscopy have been presented in Chapter 3: to reprise, Table 3.7 is reprinted below for ease of review as Table 6.1.

Micro-anatomical location of inflammation	Number of cases
free membranes only	12
mixed chorionic plate/free membranes	16
chorionic plate	28
subchorionic fibrin	68
umbilical cord	64

Table 6.1 Subclassification of cases showing histological features of ascending infection (n= 188)

6.4.1 Clinical outcomes

A number of clinical outcomes, including birth weight, Apgar score at 1 minute of age, interventional delivery, suspected neonatal sepsis and admission to the neonatal intensive care unit, were investigated for each histological subgroup. All cases showing no evidence of chorioamnionitis, regardless of gestational age, formed the control group.

For birth weight, one way analysis of variance (ANOVA) testing for birth weight showed no difference amongst the subgroups studied, as summarized in Table 6.2.

Subtype	No of cases	Mean birth weight	SD
no chorioamnionitis	933	3468	508
funisitis	64	3533	512
subchorionic inflammation only	68	3543	480
combined chorionic plate, free membranes and subchorionic inflammation	16	3721	521
chorionic plate inflammation only	28	3644	355
free membrane inflammation only	12	3408	465

Test for equality of variance: $Chi^2 = 5.85$, degrees of freedom (df) = 5, p value = 0.32.

Table 6.2. Birth weight in acute inflammation – one way ANOVA

The clinical outcomes for all cases of chorioamnionitis (present or absent) are shown in Figure 6.1 and table 6.3.

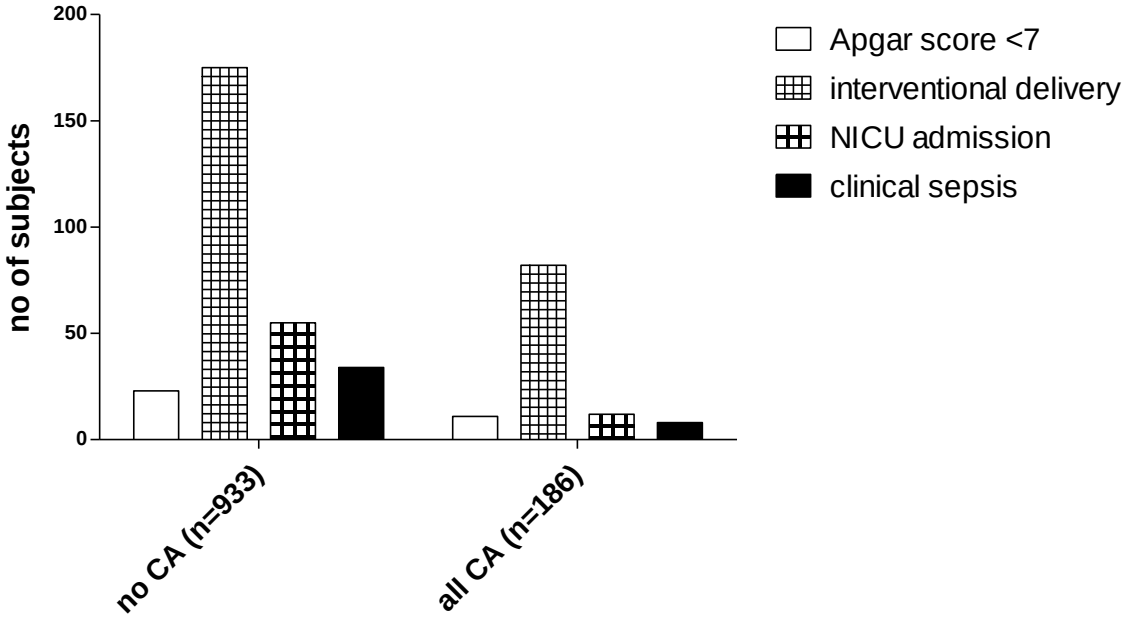


Figure 6.1. Clinical outcomes in histological chorioamnionitis (CA)

	Apgar score<7 (% cases)	**Interventional delivery (% cases)**	**NICU admission (% cases)**	**Clinical sepsis (% cases)**
no chorioamnionitis (n=933)	23 (2.5)	175 (19)	55 (5.9)	34 (3.6)
all chorioamnionitis (n=188)	11 (5.8)	82 (44)	12 (6.4)	8 (4.3)

Table 6.3. Clinical outcomes in histological chorioamnionitis (all cases)

The subgroups of chorioamnionitis examined were as follows:

- no chorioamnionitis (no CA)
- funisitis
- subchorionic fibrin inflammation (SCF inflammation)
- inflammation of chorionic plate, free membranes and subchorionic fibrin (CP, membranes, SCF)
- chorionic plate inflammation (CP only)
- free membranes (membranes only)

The clinical outcomes on analysis of chorioamnionitis are shown in Figure 6.2 and Table 6.4.

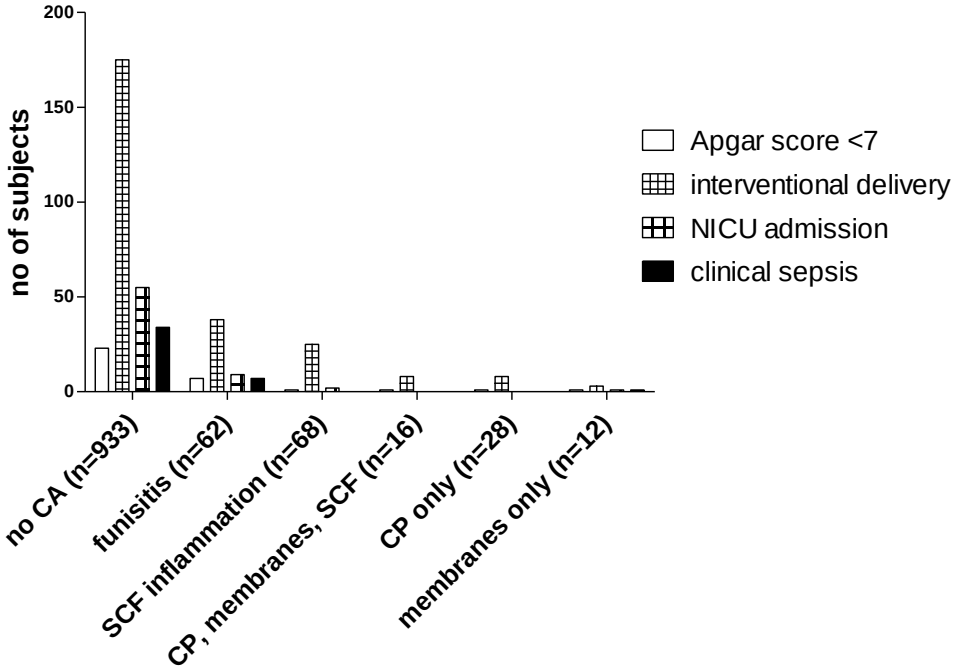


Figure 6.2. Clinical outcomes in histological chorioamnionitis subtypes

	Apgar score<7 (% cases)	**Interventional delivery (% cases)**	**NICU admission (% cases)**	**Clinical sepsis (% cases)**
no chorioamnionitis (n=933)	23 (2.5)	175 (19)	55 (5.9)	34 (3.6)
funisitis (n=64)	7 (11)	38 (61)	9 (15)	7 (11)
subchorionic fibrin (SCF) inflammation (n=68)	1 (1.5)	25 (37)	2 (2.9)	0 (0)
chorionic plate (CP),membranes, subchorionic inflammation (n=16)	1 (6.2)	8 (50)	0 (0)	0 (0)
chorionic plate inflammation only (n=28)	1 (3.6)	8 (2.8)	0 (0)	0 (0)
membrane inflammation only (n=12)	1 (8.3)	3 (25)	1 (8.3)	1 (8.3)

Table 6.4. Clinical outcomes in chorioamnionitis (subgroups)

The statistical analysis of the data presented in Figures 6.2 and 6.3 is given in Tables 6.3 – 6.8, in which the clinical associations between all chorioamnionitis and the five subgroups are examined. The control group was formed by the 933 cases showing no evidence of any acute inflammation.

For all forms of chorioamnionitis – where the study population was divided into two groups, acute inflammation present and acute inflammation absent – there were significant associations between Apgar score <7 ($p = 0.02$, OR 2.48, CI 1.15-5.14) and interventional delivery ($p < 0.001$, OR 3.41, CI 2.44 – 4.76).

When the five subgroups, addressing the significance of acute inflammation in the anatomical compartments described, were analysed, there were no significant associations between acute inflammation in the chorionic plate alone, the free membranes alone or the subchorionic fibrin alone.

Acute inflammation extending through the free membranes, chorionic plate and subchorionic fibrin was significantly associated with interventional delivery ($p = 0.006$, OR 4.3, CI 1.5 - 12.1).

Funisitis was significantly associated with all four clinical outcomes (Apgar score < 7 at 1 minute, interventional delivery, clinical evidence of sepsis and admission to the neonatal intensive care unit. The statistical analysis supporting these conclusions is given in Table 6.4

Clinical association	**Fisher's exact (2-tail)**	**Mid P exact (2-tail)**	**Odds ratio and 95% CI, mid P exact**
Apgar score <7	0.01	0.02	2.48 (1.15,5.14)
interventional delivery	<0.001	<0.001	3.41 (2.44,4.76)
admission to NICU	0.87	0.75	1.18 (0.51,2.53)
clinical evidence of sepsis	0.79	0.65	1.18 (0.51,2.53)

Table 6.5: Odds ratios and p values for all forms of chorioamnionitis (n=188) and clinical outcomes

Clinical association	**Fisher's exact (2-tail)**	**Mid P exact (2-tail)**	**Odds ratio and 95% CI, mid P exact**
Apgar score <7	0.003	0.002	5.0 (1.9,11.9)
interventional delivery	<0.0000001	<0.0000001	6.8 (4.01, 11.8)
admission to NICU	0.05	0.03	3.4 (1.3,7.6)
clinical evidence of sepsis	0.02	0.01	3.4 (1.3, 7.7)

Table 6.6: Odds ratios and p values for funisitis (n=64) and clinical outcomes

Clinical association	Fisher's exact (2-tail)	Mid P exact (2-tail)	Odds ratio and 95% CI, mid P exact
Apgar score <7	0.68	0.40	2.6 (0.12, 15.7)
interventional delivery	0.01	0.006	4.3 (1.5, 12.1)
admission to NICU	0.76	0.38	0 (0.00, 5.7)
clinical evidence of sepsis	>0.999	0.55	0 (0.00, 3.3)

Table 6.7: Odds ratios and p values for inflammation of chorionic plate, free membranes and subchorionic fibrin (n=16) and clinical outcomes

Clinical association	Fisher's exact (2-tail)	Mid P exact (2-tail)	Odds ratio and 95% CI, mid P exact
Apgar score <7	>0.999	0.665	1.5 (0.07, 8.4)
interventional delivery	0.29	0.21	1.7 (0.71, 3.9)
admission to NICU	0.38	0.19	0 (0.00, 1.8)
clinical evidence of sepsis	0.72	0.36	0 (0.00, 14.3)

Table 6.8: Odds ratios and p values for chorionic plate inflammation (n=28) and clinical outcomes

Clinical association	Fisher's exact (2-tail)	Mid P exact (2-tail)	Odds ratio and 95% CI, mid P exact
Apgar score <7	0.53	0.30	3.6 (0.16, 22.4)
interventional delivery	0.80	0.57	1.4 (0.31, 5.16)
admission to NICU	>0.999	0.44	2.4 (0.11, 14.7)
clinical evidence of sepsis	0.73	0.44	2.4 (0.11, 14.7)

Table 6.9 Odds ratios and p values for free membrane inflammation (n=12) and clinical outcomes

Clinical association	Fisher's exact (2-tail)	Mid P exact (2-tail)	Odds ratio and 95% CI, mid P exact
Apgar score <7	>0.999	0.687	0.59 (0.02, 3.25)
interventional delivery	0.001	0.009	2.52 (1.48, 4.23)
clinical evidence of sepsis	0.175	0.087	0 (0.00, 1.25)
admission to NICU	0.478	0.326	0.48 (0.07, 1.72)

Table 6.10. Odds ratios and p values for subchorionic fibrin inflammation (n=68) and clinical outcomes

6.5 DISCUSSION

There are few pre-existing studies of unselected term cohorts to offer comparison to the data presented above. As noted in Chapter 2, rates reported at term range from 10% to 35%, (38, 157, 158). The studies cited use variable definitions of chorioamnionitis and either do not report neonatal outcomes (38, 158) or report on a high-risk population (suspected meconium aspiration, premature delivery, post-mature delivery, oligohydramnios, previous stillbirth/premature delivery, maternal diabetes, maternal hypertension, substance abuse, fetal growth restriction, congenital infection and congenital anomalies) where neonatal death rates ran at 6.4% (157). One further study, published as a conference abstract only (220), examines the relationship of acute inflammation to birth weight, placental weight and gestational age in a population whose gestational age ranged from 28 – 44 weeks. The rate of funisitis in that population was reported as 18.5%. When considering the incidence of funisitis at term, 4% of one term population (n = 832) were found to have funisitis at delivery (221), while another study reports a rate of 5.1% (n = 528) (217).

In this study, histological features of ascending genital tract infection (ie an acute inflammatory cell infiltrate in one or more of free membranes, chorionic plate, subchorionic fibrin and umbilical cord) were found in 17% of cases, and the incidence of funisitis was 5.5%. Statistical analysis of the data generated by this study has found significant associations between one or more of the clinical outcomes studied for extensive placental inflammation (free membranes, chorionic plate, subchorionic fibrin) and funisitis.

For extensive placental inflammation, the association was with interventional delivery, a clinical decision reflecting concern, on the part of the clinical team caring for the labouring woman, for fetal and/or maternal wellbeing sufficient to expedite delivery – either assisted vaginal or operative. No further information is available from this study to analyse further the exact indications for interventional delivery in individual cases.

Funisitis was significantly associated with all four adverse outcomes studied. Three of these (Apgar score < 7, suspected clinical sepsis, admission to the neonatal unit) are entirely concerned with fetal wellbeing, with suspected fetal sepsis evidently also a specific indicator for possible infection. The fourth – interventional delivery – as noted above is a marker of intervention by the clinical team indicated by either fetal or maternal wellbeing, or both.

Patterns of acute inflammation in the placenta are often considered in terms of correlation with a fetal inflammatory response and a maternal inflammatory response, as discussed in Chapter 1, section 5.2. Notable in this study is the finding that the adverse outcomes identified fall into the category of a histological fetal inflammatory response.

The fetal inflammatory response syndrome is characterized by activation of the fetal innate immune system, secondary to intra-uterine infection/inflammation (116). This was originally reported to be associated with raised interleukin 6 levels in cord blood (115) but, as further studies have been undertaken, elevated levels of other cytokines are being reported (116, 222). The fetal inflammatory response syndrome is overwhelming considered in the literature in the context of preterm delivery (115, 116, 216), and is in that context associated with a raised incidence of chronic lung disease, albeit with reduced respiratory distress syndrome (215). There appears to be strong evidence associating preterm chorioamnionitis with cerebral palsy (120).

Assessing gestational-age independent effects of chorioamnionitis is more challenging, and may be difficult to achieve given continuing advances in neonatal care (223). Considering the specific question of the importance of funisitis at term – given the findings in the present study, relating funisitis to adverse clinical outcome – the literature is limited. Histological inflammation of the cord has been reported in term pregnancies in a small number of studies (217, 224), but is of uncertain significance: one of the cited

studies(217)describes significantly higher levels of cord blood interleukin 6 in preterm funisitis compared to term funisitis, and no cases of significant perinatal morbidity (congenital neonatal sepsis, suspected sepsis, respiratory distress syndrome, congenital pneumonia, bronchopulmonary dysplasia, intraventricularhaemorrhage or necrotizing enterocolitis).

The findings of this study do, however, support the view that a fetally derived acute inflammatory response is associated with what may reasonably be described as lower grade but significant perinatal morbidity. The underlying mechanisms are not addressed by this study: the controversies relating to the presence or absence of fetal infection (158) and fetal inflammatory response (217) are noted. Further investigation of the significance of funisitis at term appears warranted.

6.6 CONCLUSIONS

- 188/1119 cases showed acute inflammation
- Acute inflammation subgroups identified
    - no chorioamnionitis
    - funisitis
    - subchorionic fibrin inflammation
    - inflammation of chorionic plate, free membranes and subchorionic fibrin
    - chorionic plate inflammation
    - free membranes
- Clinical outcomes studied as discrete variables
    - Apgar score < 7
    - Interventional delivery
    - NICU admission
    - Clinical sepsis
- Statistically significant associations demonstrated between histological evidence of fetal inflammatory response and adverse neonatal outcome

CHAPTER 7: CLINICAL CORRELATION OF CHRONIC PLACENTAL VILLOUS HISTOLOGIC INFLAMMATION PATTERNS IN AN UNSELECTED POPULATION, WITH IMMUNHISTOCHEMICAL ANALYSIS

Summary:

- Cases of villitis of unknown etiology (VUE) identified
    - Subclassified according to involvement of chorionic villi and extent of inflammation
- Five specific neonatal outcomes identified
    - Birthweight
    - Apgar score < 7 at one minute of age
    - Interventional delivery
    - Admission to neonatal intensive care
    - Intra-uterine growth restriction
- Associations between villitis of unknown etiology and clinical outcomes
- Pilot immunohistochemical studies

7.1 INTRODUCTION

Chronic villitis is reported to affect between 5 and 15% of all third trimester placentas (132, 160, 225). Recognition of the lesion at less than 32 weeks gestation is virtually unknown and cases of chronic villitis at less than 32 weeks should be considered to be infectious until proven otherwise (46). Given that infection is extremely rare as a cause of chronic villitis in the third trimester (46, 226), the vast majority of cases of histologically recognised chronic villitis are described as villitis of unknown etiology (VUE).

Some do advocate very rigorous exclusion of infection in every case of VUE (132), but the diagnosis can be secured on light microscopy with a characteristic non-uniform involvement of the placental parenchyma (in contrast to the more continuous involvement in infectious villitis) (46). On light microscopy, the distribution may be focal, diffuse or basal, with mixed patterns commonly seen (46). The characteristics of areas of involvement are those of one or more terminal chorionic villi infiltrated by a mononuclear infiltrate, together with stromal expansion (160), giving the villi a somewhat "clumped" architecture on low power microscopy, as shown below.

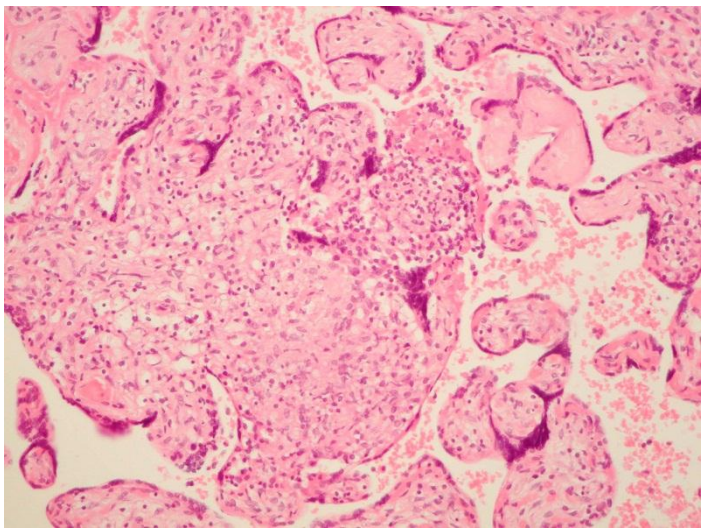


Figure 7.1. Photomicrograph of placenta, 38 weeks gestation, showing clumped chorionic villous architecture (with a mixed infiltrate of small lymphocytes and macrophages) (x 4 objective).

The clinical significance of VUE lies in multiple reports of an association between VUE and both intrauterine growth restriction (50, 159, 160, 227) and recurrent reproductive loss (46). There has been an additional suggested association with maternal obesity (160), and there are also reports of an increased incidence in ovum donation in vitro fertilization pregnancies compared with those achieved with maternal ova (228, 229) . Twin pregnancies have been shown to have considerable concordance for VUE, greater in monochorionicity than dichorionicity (230).

Recognition of VUE as a specific histological lesion on light microscopy has been followed by multiple immunohistochemical investigations to elucidate the underlying mechanisms of disease. The lymphocytic infiltrate has been demonstrated, relying on differential sex chromosome expression in male fetuses, to be of maternal origin (231, 232). HLA-DR expression, by macrophages within areas of villitis, has been reported on frozen section immunofluorescence (233). The lymphocytic population has been shown to be composed of predominantly CD8+ T cells (47, 234, 235). Syncytiotrophoblast has been shown to express intercellular adhesion molecule -1 (ICAM-1) (236). The T cell population has been demonstrated to have a T regulatory (Treg) phenotype. In the same study, HLA-DR expression by macrophages in VUE was confirmed but no HLA-DR expression by trophoblast demonstrated (237).

In this study, the outcomes recorded are those from a single episode of ongoing intrauterine pregnancy and so no data are available to address the question of recurrent pregnancy loss. The main focus of this part of the study is thus to address the reported association between VUE and intrauterine growth restriction, and to report on a pilot study of a panel of immunohistochemical markers addressing mechanisms of disease in VUE.

7.2 METHODS

Recruitment to the study, recording of clinical data, microscopic and microscopic placental examination and immunohistochemical methods used are recorded in Chapter 2. The placental sampling protocol used in this study has previously been reported to support maximum detection of chronic villitis lesions (50).

7.3 STATISTICAL ANALYSIS

Fisher's exact and mid P values were calculated, together with odds ratios and 95% confidence intervals: please see Chapter 2.

7.4 RESULTS

Sixty three cases of VUE were identified in the cohort of 1119, 5.6% of cases. This rate is at the lower end of, but within the range of, the spectrum of rates reported in other cohorts . Twenty cases were categorised as high-grade, 23 as low-grade and 20 as low-grade multifocal. All cases of VUE identified fell within the gestational age range 37-41^{+} weeks and so the control group of all non-VUE cases was restricted to the same gestational age range. There was no statistical significant difference between the number of cases with villitis of unknown etiology and controls for each completed gestational week.

	VUE (%) n=63	**Non VUE (%) n=1019**	**p value**
37 weeks	2 (3)	57 (6)	0.41
38 weeks	5 (8)	135 (13)	0.30
39 weeks	16 (25)	269 (26)	0.99
40 weeks	17 (27)	290 (28)	0.92
41 + weeks	23 (36)	268 (26)	0.10

Table 7.1. Villitis of unknown etiology vs controls by gestational week

Analysis of growth and neonatal outcomes

As noted above, the case series previously reporting clinical outcomes in VUE report a consensus in favour of VUE being a lesion that is associated with intra-uterine growth restriction. This effect was not apparent in this cohort, in which both one way ANOVA testing (see table 7.1) and comparison of rates of IUGR (see Figures 7.2 and 7.3, and Tables 7.2 – 7.5) showed no evidence of a statistically significant difference in birthweight or IUGR. When considered in subgroups (high-grade VUE, low-grade focal VUE and low-grade multifocal VUE) there was also no demonstrable difference between the birthweights recorded in each group. Z score-based analysis of birth weights, correcting for gestational week, did not demonstrate any significant difference between VUE and control cases (see Figure 7.4).

Subtype	No of cases	Mean birth weight	SD
no VUE	1019	3516	478
high-grade VUE	20	3537	311
low-grade focal VUE	23	3391	463
low-grade multifocal VUE	20	3318	561

Test for equality of variance:
$Chi^2 = 6.39$, degrees of freedom (df) = 3, p value = 0.09
Table 7.2. Birth weight in VUE – one way ANOVA

Further analysis of clinical outcomes which might be expected to be affected by an intra-uterine environment presenting a degree of chronic fetal challenge – Apgar score at one minute, interventional delivery and admission to a neonatal intensive care unit (NICU - was undertaken. This also showed no evidence of adverse outcome in the setting of VUE for both all forms of VUE considered together and on analysis of subtypes. This information is also presented in Figures 7.2 and 7.3, and Tables 7.2 and 7.3.

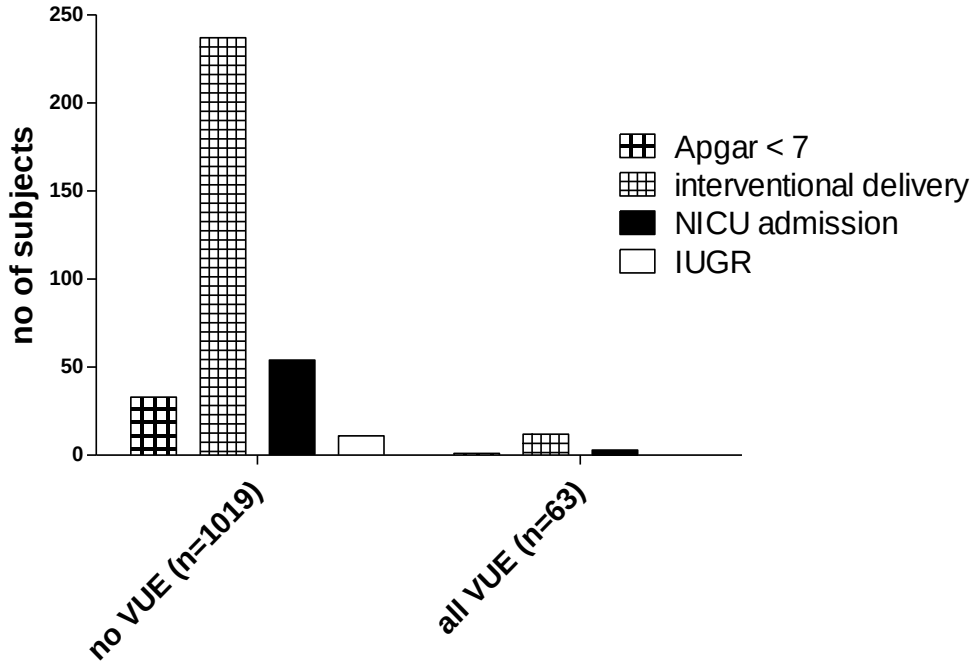


Figure 7.2. Clinical outcomes in villitis of unknown etiology

	Apgar score <7 **(% cases)**	**Interventional delivery** **(% cases)**	**NICU admission** **(% cases)**	**Intra-uterine growth restriction (% cases)**
No VUE (n=1019)	33 (3.2)	237 (23.2)	54 (5.3)	11 (1.1)
all VUE (n=63)	1 (1.6)	12 (19)	3 (4.7)	0 (0)

Table 7.3. Clinical outcomes in villitis of unknown etiology (all cases)

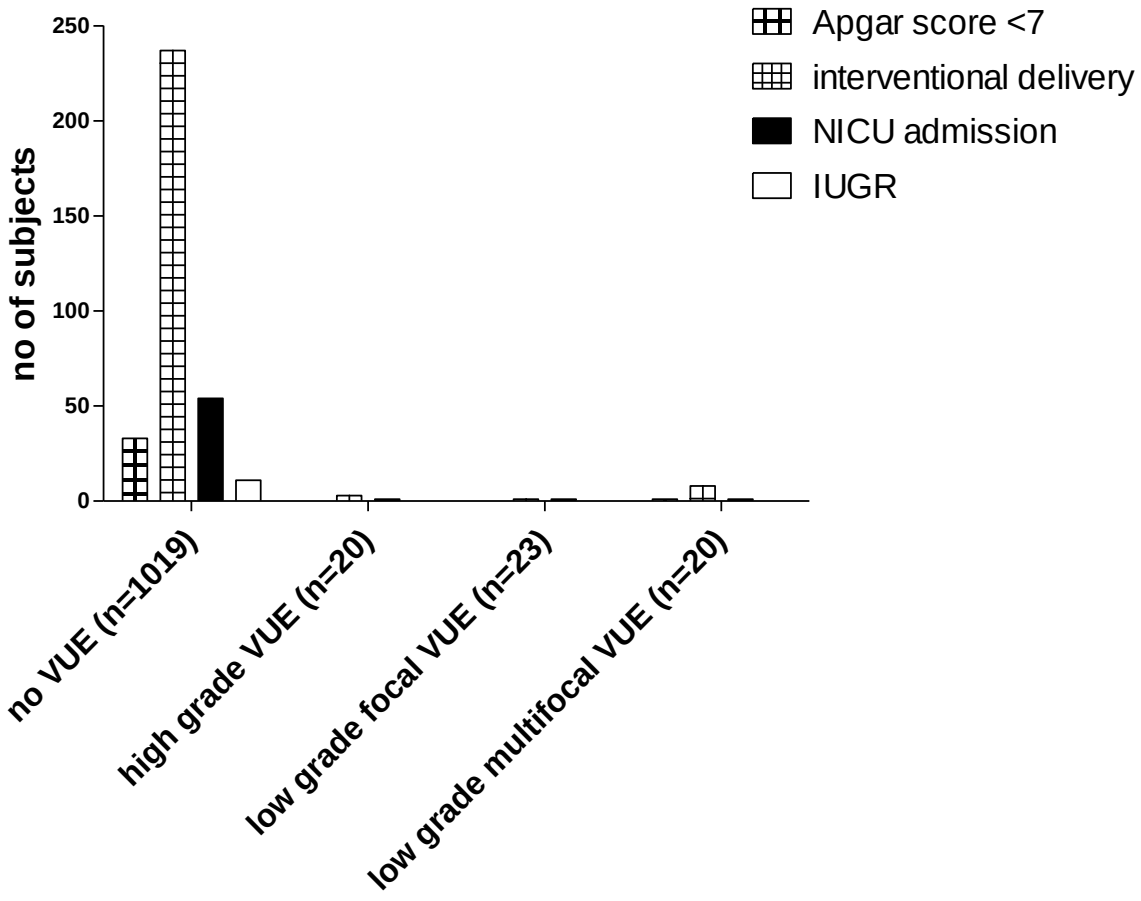


Figure 7.3. Clinical outcomes in VUE subtypes

	Apgar score <7 (% cases)	**Interventional delivery (% cases)**	**NICU admission (% cases)**	**Intra-uterine growth restriction (% cases)**
No VUE (n=1019)	33 (3.2)	237 (23.2)	54 (5.3)	11 (1.1)
High-grade VUE (n=20)	0 (0)	3 (15)	1 (5)	0 (0)
Low-grade focal VUE (n=23)	0 (0)	1 (4.3)	1 (4.3)	0 (0)
Low-grade multifocal VUE (n=20)	1 (5)	8 (40)	1 (5)	0 (0)

Table 7.4. Clinical outcomes in villitis of unknown etiology (subgroups)

Clinical association	**Fisher's exact (2-tail)**	**Mid P exact (2-tail)**	**Odds ratio and 95% CI, mid P exact**
Apgar score < 7	0.78	0.51	2.1 (0.39, 44.0)
Interventional delivery	0.18	0.14	1.59 (0.86, 3.12)
NICU admission	>0.99	0.91	1.12 (0.38, 4.64)
IUGR	>0.99	0.51	0.0 (0.00, 5.11)

Table 7.5. Odds ratios and p values for all VUE (n=63) and clinical outcomes.

Clinical association	**Fisher's exact (2-tail)**	**Mid P exact (2-tail)**	**Odds ratio and 95% CI, mid P exact**
Apgar score < 7	>0.99	0.52	0.00 (0.00, 5.04)
interventional delivery	0.57	0.41	0.58 (0.13, 1.84)
NICU admission	>0.99	0.95	1.06 (0.19, 22.7)
IUGR	>0.99	0.81	0.00 (0.00, 5.04)

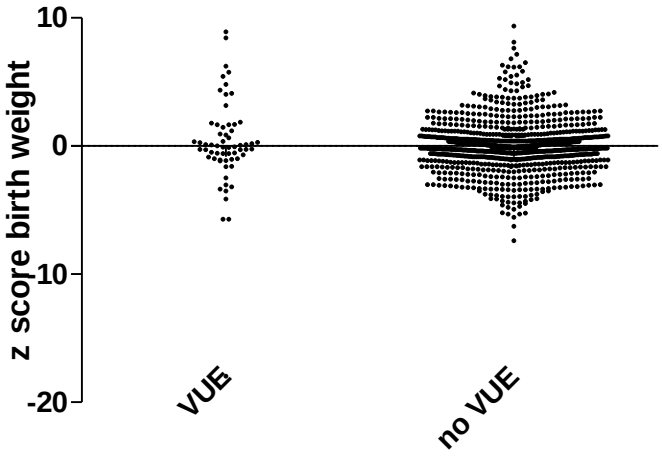
Table 7.6. Odds ratios and p values for high-grade VUE (n=20) and clinical outcomes.

Clinical association	Fisher's exact (2-tail)	Mid P exact (2-tail)	Odds ratio and 95% CI, mid P exact
Apgar score < 7	>0.99	0.76	1.35 (0.06, 7.68)
interventional delivery	0.03	0.02	0.15 (0.007, 0.81) *
NICU admission	>0.99	0.94	0.81 (0.04, 4.56)
IUGR	>0.99	0.78	0.00 (0.00, 14.6)

Table 7.7. Odds ratios and p values for low-grade multifocal VUE (n=20) and clinical outcomes. (*favours control group)

Clinical association	Fisher's exact (2-tail)	Mid P exact (2-tail)	Odds ratio and 95% CI, mid P exact
Apgar score < 7	0.98	0.62	1.57 (0.08, 9.0)
interventional delivery	>0.99	0.98	0.98 (0.40, 2.22)
NICU admission	>0.99	0.95	0.94 (0.04, 5.3)
IUGR	>0.99	0.81	0.00 (0.00, 19.0)

Table 7.8. Odds ratios and p values for low-grade focal VUE (n=23) and clinical outcomes.



unpaired t test: p=0.70

Figure 7.4. z scores (birthweights), VUE vs controls

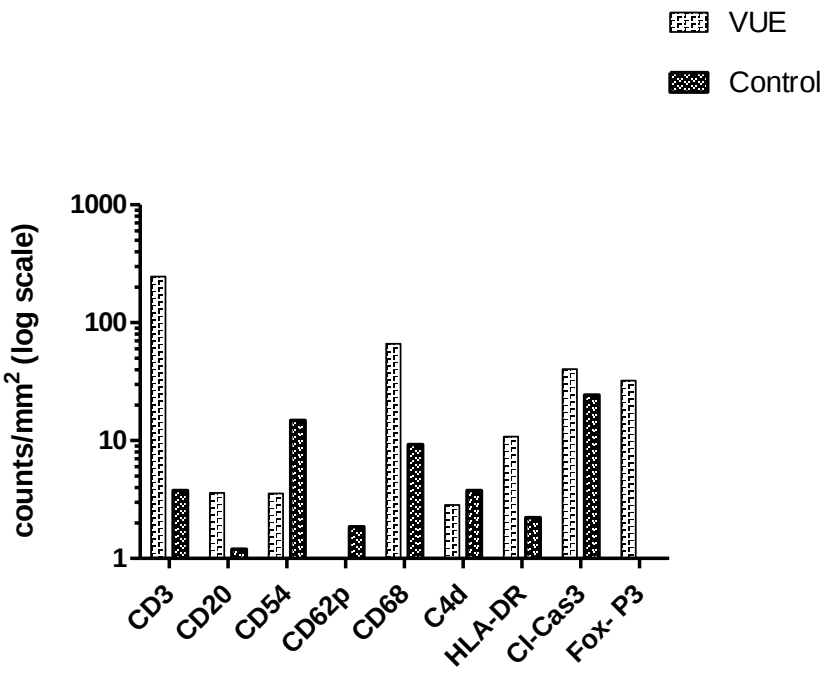
Immunohistochemical studies

Immunohistochemical studies were carried out on two sets of micro-arrays – VUE and controls, with 20 cases in each set. For ease of reference, table 5.4 is reproduced below as Table 7.6.

Primary antibody	Cell type/antigen
CD3	T cell
CD20	B cell
HLADR	Cell surface class II major histocompatibility complex
CD68	Macrophage/monocyte series
C4d	Complement activation
cleaved caspase 3 (Cl-Cas3)	Apoptosis marker
ICAM (CD54)	Leukocyte/endothelial transmembrane adhesion molecule
P selectin (CD62P)	Activated endothelial/activated platelet transmembrane adhesion molecule
Fox P3	T regulatory cell (TREG) phenotype

Table 7.9. Immunohistochemical panel: target cell type/antigen

The outcomes of the immunohistochemical studies are shown in graphical and tabular form in Figure 7.5. The infiltrating inflammatory cell population showed increased expression of CD3, CD20, CD68, HLA-DR and Fox-P3 compared with controls. CD3, CD 20 and CD 68 had a membranous pattern of staining. HLA-DR also had a cell surface distribution, and appeared possibly to be localised to syncytiotrophoblast as well as the surface membranes of histiocytes, although the overlap between cell types in areas of dense inflammatory infiltrate was considerable. Fox-P3 had a nuclear staining pattern. CD3 and HLA-DR immunostaining is illustrated in Figure 7.6.



	VUE			Control		
	Mean	**SD**	**N**	**Mean**	**SD**	**N**
CD3	246.00	176.00	20	3.76	2.93	20
CD20	3.60	3.30	20	1.20	1.36	20
CD54	3.56	3.30	20	14.80	34.20	20
CD62p	0.38	0.86	20	1.86	2.49	20
CD68	66.40	91.60	20	9.25	15.10	20
C4d	2.85	4.36	20	3.76	13.80	20
HLA-DR	10.80	7.37	20	2.22	3.18	20
Cl-Cas3	40.40	34.10	20	24.30	34.60	20
Fox- P3	32.20	48.20	20	0.00	0.00	20

Figure 7.5. Immunohistochemical analysis:
VUE vs controls

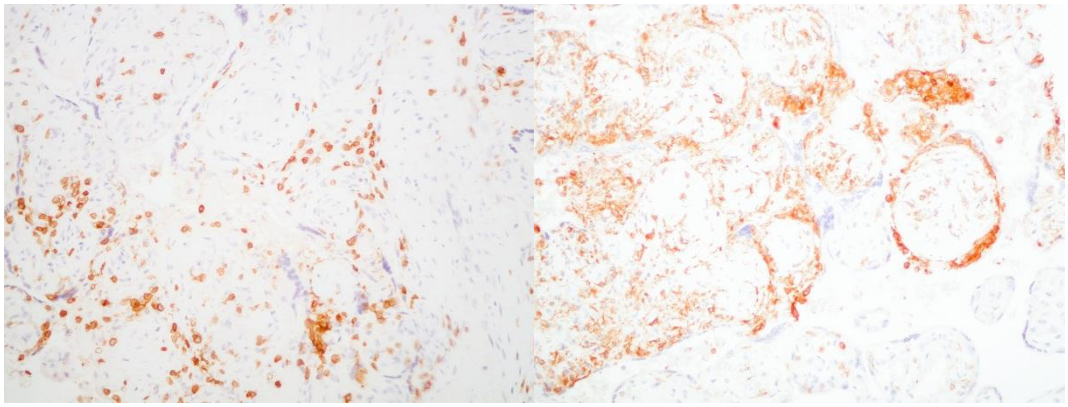


Figure 7.6. Immunohistochemical expression of CD3 (left) and HLA DR (right) in a focus of villitis of unknown etiology (x 10 objective)

7.5 DISCUSSION

The results in this part of the study were perhaps the most surprising in the study overall, given the weight of findings in previous publications. The underlying reasons for this variation may not be entirely identifiable, but it is possible to speculate on some possible contributory factors to the differences between this study and those previously published.

Apart from the small series of 50 cases dating from 2007-2008 (161), the three large case series which form the basis of the epidemiological evidence for the clinical outcomes seen in association with VUE date from 1976-1978 (159), 1979-1980 (50) and 1995-1997 (160). Clinical management regimes will evidently have evolved in the interval between those large case series (ranging from 529 to 7505 cases) and the present. Small for gestational age fetuses are likely to be recognised at an early point in pregnancy (238, 239), with subsequent clinical management directed towards delivery of the fetus at the most advantageous point for subsequent neonatal wellbeing. With the advances in neonatal care since the conclusion of the last large case series examining VUE and growth restriction, the possibility exists that a subgroup of fetuses clinically affected by VUE is being delivered earlier than was the case ten or twenty years ago. As previously noted in the analysis of data from this study, there was a recruitment bias in the study design in that, in retrospect, favoured recruitment of low-risk women delivering at term. Those women presenting as an emergency, or those women delivering semi-electively pre-term, would have been less likely to be recruited in routine ante-natal clinics and less likely to have been approached about the ongoing research study in an emergency setting. It may thus have proven that this study selected out a number of pregnancies affected by VUE.

The findings in this cohort could, however, be consistent with two further interpretations: this cohort may represent a variant with outcomes (in this study) at the better end of the range or VUE may not, in fact, represent as serious a risk to fetal growth and well-being as has previously been thought.

Immunohistochemical analyses were in keeping with previously reported studies (77, 240). As has recently been reported, a proportion of the inflammatory cell infiltrate has a Fox-P3 positive T regulatory cell phenotype (237); a similar pattern was found in this cohort. There is thus no evidence that the phenotype of the inflammatory cell infiltrate in this study is substantially different from that reported in previous cohorts.

In conclusion, this pattern of placental inflammation, which has previously been considered as incontrovertibly abnormal, requires additional study. An extended review of the data from previous studies (beyond the scope of the present study) is required, together with continuing investigation, possibly in the form of nested case control studies, into the significance of villitis of unknown etiology in the context of modern obstetric practice.

7.6 CONCLUSIONS

- 63/1119 cases showed villitis of unknown etiology (VUE)
- VUE subgroups identified
    - High-grade VUE
    - Low-grade focal VUE
    - Low-grade multifocal VUE
- Extended analysis of growth undertaken
    - One ANOVA of mean birth weights for gestation-matched controls
    - Identification of IUGR pregnancy rates in VUE and controls
    - z score analysis of VUE and controls corrected for gestational week
- Clinical outcomes studied as discrete variables
    - Apgar score < 7
    - Interventional delivery
    - NICU admission
    - Intra-uterine growth restriction
- No statistically significant associations between VUE and growth or adverse neonatal outcomes
- Immunohistochemical studies
    - CD3, CD20, CD68 rich infiltrate
    - HLA-DR expression on histiocytes
    - HLA-DR expression on syncytiotrophoblast
    - T regulatory cell phenotype identified

CHAPTER 8: DISCUSSION

Summary:

- Study design
- Placental macroscopic examination
- Placental histological examination
- Modern obstetric and neonatal care
- Study population

8.1 REVIEW OF FINDINGS IN THIS STUDY

This study has addressed the question of which placental lesions are significant for adverse perinatal outcomes in a low-risk population delivering at or near to term. Following initial analysis of the data, four specific lesions were identified for further evaluation: umbilical cords with coiling > 90th or < 10th centile; placental infarction; villitis of unknown etiology and acute inflammatory cell infiltrates consistent with ascending genital tract infection.

There are considerable numbers of publications suggesting that placental examination is, in many cases, the key to understanding adverse events in pregnancy and the neonatal period (28, 110, 111, 113, 241-244), and many studies – as reviewed extensively in the preceding chapters – suggest that specific macroscopic and microscopic lesions are positively associated with significant clinical issues.

Analysis of the data obtained from this study shows no association between the clinical outcomes studied and umbilical cord coiling > 90th or < 10th centile, chronic villitis of unknown aetiology or placental infarction.

Funisitis at term and extensive acute inflammation of the chorionic plate were identified as being associated with a number of neonatal events. Significant associations between other histological patterns of ascending genital tract infection and adverse neonatal outcomes were not found. It should, however, be noted that, even for funisitis and chorionic plate inflammation in this study, the clinical neonatal care provided appeared sufficient to result in discharge to home of a healthy infant. Long-term follow-up did not form part of this study protocol.

Specific reasons for the variation between present findings and previously published studies have been addressed in individual Chapters but a number of common key themes emerge for review: effective study design, criteria for macroscopic examination of the placenta, criteria for histological examination of the placenta, modern obstetric and neonatal care, and the nature of the population in this study.

Design of this study & design of previous studies in this field

Cohort studies are designed to study incidence, causes and prognosis (245). In this study, the incidence and short-term outcome of a number of specific placental lesions were studied. This format was probably the most realistic methodology that could have been selected to investigate the research question asked and, although time-consuming and relatively expensive, has provided robust information about 1119 subjects delivering at or close to term. As has been noted, a large number of studies reporting positive data in this field are retrospective case-control studies.

While the recruitment bias in the present study (tending to recruit low-risk women at or near term) has been recurrently acknowledged, the risks of bias inherent in smaller, retrospective case control studies are large and rarely explored, with both sampling bias and retrospective analysis bias introduced into case control studies from the outset (245) . There is also no means of establishing the existence of any existing research data supporting the findings in this study, given the acknowledged tendency within the research community to publish only positive results (246).

When attempting to review the results of previously published work in this area, the effects of clinical heterogeneity should be considered. This is of critical importance in the construction of a systematic review and meta-analysis, but even without undertaking a formal exercise such as this, the presence of clinical heterogeneity when comparing other studies cannot be overlooked. Clinical heterogeneity is introduced when there are differences amongst patient selection, baseline morbidity, definitions of disease, clinical interventions and duration of follow-up (247, 248). The literature reviewed to support this study has indeed shown considerable heterogeneity and must be at least contributory to the variation in findings between this study and others.

Criteria for macroscopic examination of the placenta

There continues to be disagreement relating to the most effective method of examining a placenta macroscopically. It is said that placental infarcts - particularly fresh infarcts – are more easily identified visually post-fixation, although palpable as a firm lesion when unfixed. Areas of downstream villous avascularity are also said to be difficult to detect in the unfixed state (3). Placental weight is, however, increased by around 7% following

fixation (249). On balance - given the need for consideration of fetal:placental weight in this study - examination of the unfixed placenta was preferred.

The potential for interobserver error in the macroscopic examination of the placentas in this study certainly existed, as two prosectors undertook placental examination during the course of recruitment. One prosector was a consultant histopathologist (FAJ) while the other was a research fellow in obstetrics (SP). SP was provided with extended training by FAJ in placental examination, at the outset of the study, including a number of supervised sessions. Any error in macroscopic placental examination is thus, in this study, likely to be systematic, given that the criteria for recognition of abnormality were essentially those of one prosector (FAJ).

Criteria for histological examination of placenta

There are a number of reporting schedules for various placental lesions. In this study, histological diagnoses of specific conditions were made with as much reference to previously published descriptions as was possible. In particular, all possible lesions, no matter how low-grade, were identified by re-screening of cases which would, under routine clinical practice in the laboratories involved in this project (Addenbrooke's and Great Ormond Street), be reported as normal. Moderation of diagnostic standards used was undertaken at the outset of the project, by dual screening of approximately 10% (100/1119) of cases by two consultant histopathologists. (FAJ and NJS).

Modern obstetric and neonatal care

Real-time scanning and progressive improvements in image quality have led to the generation of validated fetal growth charts (250, 251), permitting earlier and more accurate detection of fetal growth impairment (252). Growth charts have continued to be reviewed and revised (253-256). While the effectiveness of current screening for growth restriction by tracking fundal height (257, 258) and routine Doppler/ultrasound (259, 260) continues to be debated, fetal biometry and Doppler flow are the mainstay of surveillance of fetal growth and wellbeing in detected cases of fetal growth impairment (257).

Other issues relating to clinical care, such as birth during weekday normal working hours (261, 262) and place of birth (home, midwife-led birth unit, obstetric units) (151, 182), are associated with variations in perinatal mortality and morbidity.

Neonatal care has advanced rapidly in recent years (263), but while neonatal mortality has been carefully tracked and demonstrated to be falling (7.7/1000 in 1980 to 2.9/1000 in 2010) (264), large population-based studies reporting incidences and trends in neonatal morbidity are rare.

Source: Office for National Statistics

Figure 8.1. Trends in neonatal mortality
England and Wales, 1980 - 2010

Studies describing neonatal morbidity tend to report on long-term outcomes following premature birth. The target population of the present study was a low-risk term cohort, with a low frequency of adverse neonatal outcomes. It is acknowledged that tracking neonatal outcomes data are a challenging area (265). A few studies exist reporting outcomes of low-risk term infants: one records an incidence of primary outcome events including birth trauma, severe morbidity (meconium aspiration, encephalopathy) and mortality of <0.5% (151), while in another the incidence of neonatal intensive care unit admission was between 5.2% (term vaginal births) to 9.8% (term elective caesarian birth) – a rate much higher than historical UK admission rates of 0.3% (266).

In summary, there is good evidence from the obstetric literature that clinical surveillance of ongoing pregnancy continues to improve, and the neonatal mortality statistics available

would tend to suggest that modern practices in both obstetric and neonatal care have improved perinatal outcomes. On the limited evidence available, the rate of relatively low-grade neonatal morbidity appears low. It is also noted that, as might be expected, factors other than the intrinsic wellbeing of the feto-placental unit affect neonatal outcomes in low-risk populations, including such variables as place and time of birth.

These observations are important and relevant in the context of the present study. Literature dating from a number of years ago may report outcomes in clinical cases which are now managed more pro-actively, altering pregnancy outcome in the setting of a placental lesion previously associated with adverse outcomes. While the contribution of improving clinical care to the largely good perinatal outcomes in this study - versus the possibility that lesions previously suspected of inducing significant pathology are in fact completely innocuous - is difficult to assess, it must remain under active consideration when considering the implications of the findings in this study.

Study population

The composition of the population in this study was overwhelmingly low-risk and at term: this is, as discussed elsewhere, rare in studies evaluating of pregnancy outcomes. Management of low-risk pregnancy in the United Kingdom is minimally interventional, with most women being offered a late first trimester dating scan, serum screening and a midtrimester anomaly scan (239), the regime thus offered to the women in this cohort.

High-risk women are identified either by antecedent clinical history or events in an ongoing pregnancy. The management of high-risk pregnancy has become more interventional in more recent years and this subgroup of pregnant women will thus be offered a more complex management plan as discussed above (267). Specific events conferring high-risk on a current ongoing pregnancy are highly variable (199, 203, 267-269), and include the identification of fetal anomalies not otherwise addressed in this study (270-272). High-risk women did not form a viable study group in the current population, given that recruitment was focused on routine antenatal clinics and relatively uncomplicated term deliveries.

Placing the outcomes of this study into the context of those for other studies reporting low-risk population outcomes does, however, show (as far as can be determined) comparable

rates of obstetric and neonatal events between this study population and other term populations (see data summarized in Chapter 3). Neonatal primary outcome measures in large population studies are very often set at the severe end of the spectrum (perinatal death, birth injury, low birth weight) (150, 151). Placing this study's findings in context has thus relied more on individual studies reporting a smaller number of outcomes. Where possible – eg birth weights – nationally reported statistics were employed (see Chapter 3).

A very large number of studies report specifically on placental lesions in the context of the clinical outcome of preterm deliveries (31, 44, 103, 106, 214, 273-280), and providing a comprehensive review of them is beyond the scope of the present study. Reference to studies of preterm placentas is, however, relevant to this study of term infants in that placental findings in the preterm population - such as florid acute inflammatory lesions and maternal vascular lesions seen in the setting of pre-eclampsia - are associated with adverse preterm outcomes. Some of the lesions reviewed in this study, specifically acute inflammatory lesions and placental infarction, are morphologically reminiscent of those correlated with severe adverse events in the preterm infant, but appear in this study to show more benign clinical associations. Any inclination to extrapolate directly from preterm to term placentas does not, on the present evidence, appear justified.

Conclusion

This study reports on the clinical and placental findings in a cohort of 1119 women, predominantly low-risk and delivering at term. Analysis of specific placental lesions, previously identified by other researchers as conferring significant clinical risk for adverse perinatal outcome, was undertaken. The infants within this cohort were largely able to withstand any adverse effects of the placental lesions examined.

The possible underlying reasons for this are considered above, and may be multifactorial. The design of previously reported studies may have introduced a considerable degree of bias into their conclusions. While it is important not to overlook any possible flaws in the design of the current study, there is no reason to believe that the methodology of either macroscopic or microscopic examination has introduced systematic bias into this study. It is acknowledged that the design of the current study did introduce recruitment bias in favour of low-risk term pregnancies – but the rate of specific placental lesions in this

population was comparable with other studies, and so the relative absence of adverse outcomes in this study continues to require explanation.

The term infant, with more mature systemic physiology and a larger placenta with increased reserve, may prove be much less vulnerable to the impact of a lower grade placental abnormality. The mothers in this study were predominantly healthy, low-risk women. The context, in this study, of increasingly advanced obstetric and neonatal care may have contributed to the observed outcomes, by overcoming the potentially adverse effects of the placental lesions present.

In the last analysis, however, it may prove simply to be the case that the placental lesions examined in this study are largely innocuous. This study is thus of importance in contributing to a better understanding of the pathophysiology of adverse term neonatal outcome, and challenges a tendency in both medicolegal and clinical practice to attribute a range of adverse outcomes to underlying placental pathology (281). The future directions of further investigations which may be undertaken in this field are considered in the following chapter.

CHAPTER 9: FURTHER WORK

Summary points:

- Further population-based studies
- Expansion of systematic review format
- Laboratory-based tissue analysis
    - Immunohistochemistry
    - Proteomic analysis
    - microRNA studies
    - Evaluation of biomarkers

9.1 INTRODUCTION

Inevitably, at the conclusion of any research study, some initial questions remain unanswered, while the results of the study itself raise further issues for exploration. This study had many components and the future directions of new studies which could develop from this work are set out below.

9.2 FURTHER POPULATION-BASED STUDIES

As has been discussed extensively in the preceding chapters, a fundamental objective of this study was to determine the incidences of a number of specific placental lesions and link these lesions with clinical outcomes. It has thus been demonstrated that funisitis at term is a potentially significant lesion for neonatal well-being, even in unselected low-risk pregnancies. Conversely, and in contrast to a number of previously published studies, this study did not identify a strong link between high-grade villitis of unknown etiology and neonatal outcomes, including birth weight.

A separate, but also highly important, learning point from this project is that daily collection and examination of large numbers of placentas from low-risk deliveries represented a huge logistical task, in many ways greater than had been anticipated at the inception of the study. Given, however, that the work is complete, and the incidence rates for a range of histological lesions for the Cambridge population delivering at the Rosie Hospital is now defined, further large cohort based studies in Cambridge are unlikely to be significantly useful: rather, opportunities for more targeted placental examination now exist.

In an era of rapid discharge of low-risk mothers and infants from delivery units, readmission with suspected neonatal sepsis, or indeed less specific events such as readmission with poor feeding/neonatal dehydration/poor weight gain, are events which both undermine early mother and baby attachment and represent cost pressure in a health care system which remains under considerable financial strain. Care pathways should include rapid and simple investigations which can provide early identification of potential problems. It would, for example, be extremely interesting, and potentially very useful, to investigate rates of funisitis in early readmissions following discharge home. More generally, then, design of further study protocols with defined clinical outcomes examined in a nested case control format, would be both cost efficient and of potential benefit to new

mothers and infants by assisting those who care for them to make more appropriate and timely decisions relating to their wellbeing.

9.3 EXPANSION OF SYSTEMATIC REVIEW FORMAT

There was no certainty, at the discussion stage of drawing up this study, that application of the systematic review format to macroscopic and microscopic placental findings – linked to clinical outcomes – would be fruitful. While very familiar to practicing clinicians, to whom the Cochrane review database is a day-to-day resource which assists in planning treatment plans for individual patients, systematic review analysis is not well established in histopathology. This format has, however, been extremely useful, not least in supporting extended literature searches for all topics included in this study. The core concept applied – ie, that the more familiar treatment intervention can be replaced by considering the suspected pathological event as the "intervention" for analysis – has been shown to be workable. Also noted more specifically in the systematic review of cord coiling was the degree of heterogeneity amongst previously published studies, thus highlighting the requirement for high-quality cohort studies with reduced risks of bias.

Having piloted the format for analysis of cord coiling outcomes, the next step is to examine other pathological lesions in the same manner, assuming sufficient studies exist to support the format.

Detailing the outcomes recorded for the Cambridge cohort in the setting of a relevant systematic review is the most likely next stage of investigation to follow on from this project. To this end, it is pleasing to report that Professor Peter Russell (Melbourne), one of the earliest investigators in the field of villitis of unknown etiology, has kindly sent his original research books and papers to the present candidate in May 2012, to support a systematic review in this particular field.

9.4 LABORATORY-BASED TISSUE ANALYSIS

Immunohistochemistry

Extension of the pilot immunohistochemical studies undertaken is indicated, with particular focus on immune system activation and apoptotic pathways. As discussed below, these pathways, once established by immunohistochemistry to be activated in specific

placental abnormalities, may subsequently be further investigated by protein and nucleic acid analysis.

Proteomics

A proteome (282, 283) is a set of proteins expressed by a given genome in a specific time and space. This definition reflects the unique properties of a proteome, the composition of which is regulated by both intrinsic and extrinsic factors as summarized in Figure 9.1

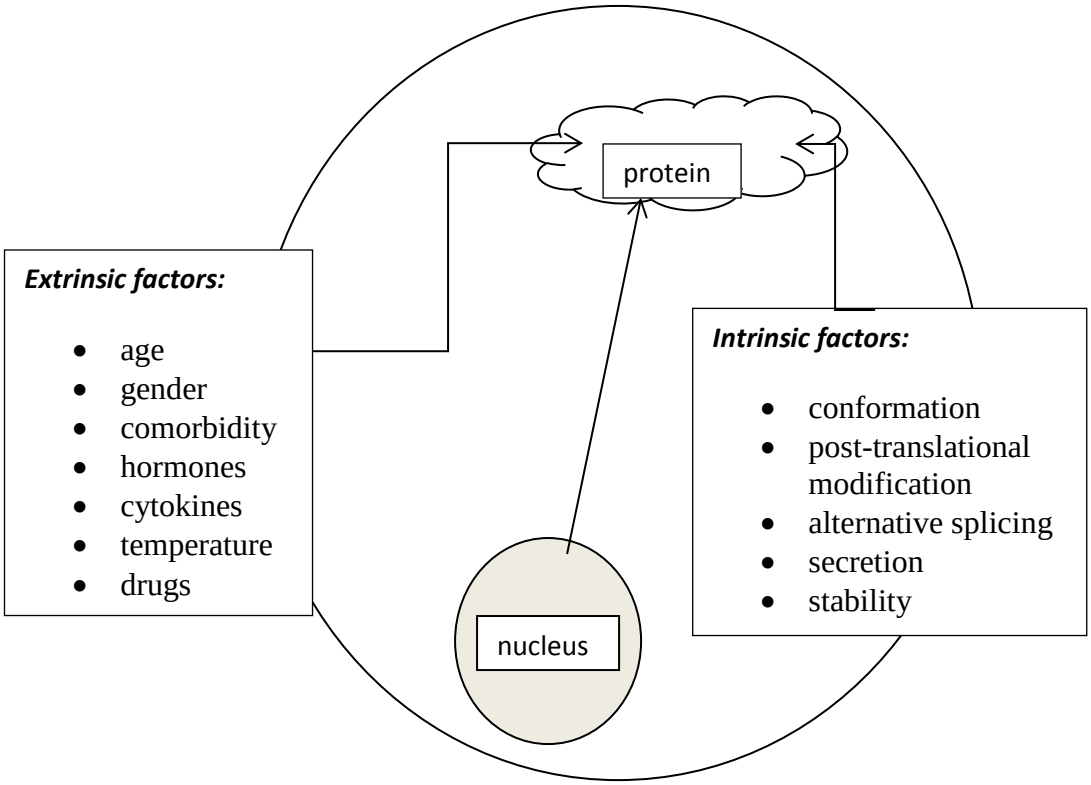


Figure 9.1. Diagram of cell summarizing contribution of extrinsic and intrinsic factors modulating proteome expression.

A good biomarker for a specific disease should have several characteristics, as given in Table 9.1. No equivalent of PCR amplification exists in the proteomic field, and, for a number of clinical conditions, the concept of a single specific biomarker for disease has

been superseded by the recognition that a panel approach to analysis of biomarkers is much more specific and sensitive (284).

Specific to organ/disease process
Segregates to clinical entities
Not present (or not increased) in diseases sporadically associated with milder forms of the disease
Not present in healthy individuals
Present in sufficient quantity in blood for reliable measurement
Stable in blood (serum or plasma) with storage

Table 9.1. Minimum criteria of a biomarker

Proteomic studies most commonly report findings validated in biological fluids (most commonly serum and plasma) with the biomarker(s) selected indicating damage to the organ of interest, eg myocardium in ischaemic heart disease (285) or cell type in malignant disease (284). The alternative approach is that of primary identification of biomarkers within tissue with secondary validation in serum or other biological fluid analysis.

A small number of studies of proteomic analysis exist in the analysis of placental disease. Sampling of amniotic fluid for proteomic analysis of inflammatory markers has, for example, been reported in the context of histological chorioamnionitis and villous tissue has been analysed in a small series of early pregnancy loss (286, 287).

Future nested case control studies as described above provide the opportunity for extended immunohistochemical panels being translated to more complex proteomic analysis. In this context, newly described methodologies for analysis of the proteome in tissue sections has recently been reported (288).

MicroRNA studies

Small, non-coding RNAs are described as microRNAs (miRNAs): as non-coding RNAs, no protein product is transcribed. Regulation of miRNAs is effected by post-transcriptional processing of precursor transcripts to regulatory RNAs – a reduction from around 60 nucleotides to around 20 is common (289). Key miRNA processing genes, by regulating the conversion of precursor transcripts to active miRNAs, control the levels of active

mRNAs within the nucleoplasm – the Dicer gene, for example, is an important miRNA processing gene (290). Processing results in variations of levels of miRNA compared with their precursor transcripts, and it is variations in miRNA levels which appear to be linked with specific disease processes. The role of miRNAs in gene transcription is summarized in Figure 9.2 – as indicated, the target of miRNAs is messenger RNA (mRNA), which is either inhibited or destroyed in the downstream pathways.

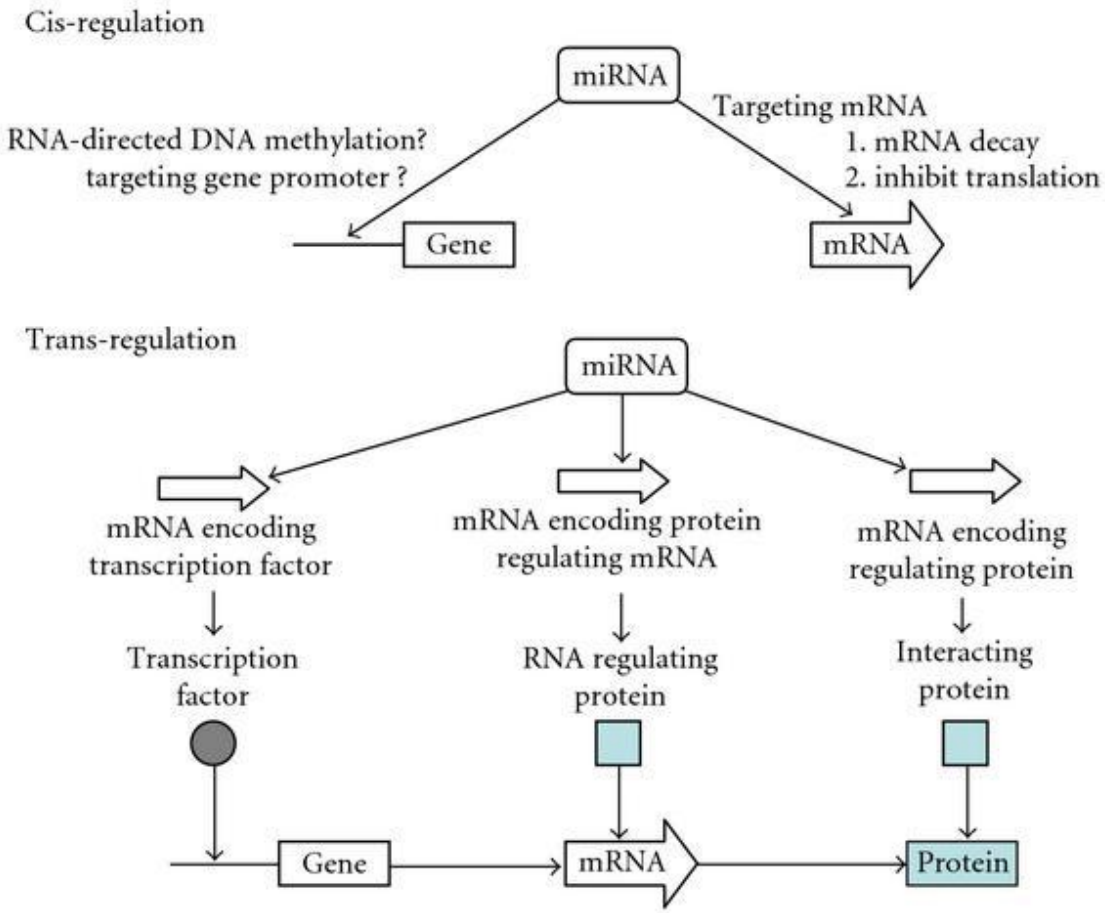


Figure 9.2. The role of miRNAs in gene transcription .

Detection of miRNAs has become technically much more feasible in the last decade, with microarray analysis, deep sequencing, realtime PCR, Northern blotting and in situ hybridization all established techniques. The key aspects of each approach are given in Table 9.2.

microRNA detection method	methods	key points
microarray	• RNA extracted from cells or tissues • direct labeling of miRNA (fluorescent nucleotides)	• does not support quantitative detection • probes must allow for short length of miRNAs
deep sequencing	• RNA isolated and fragmented • cDNA synthesis • ligation of adaptor (fluorescent oligonucleotide) to fragments • amplification of millions of sequence (automated) • ≈ 20 nucleotide sequences selected and mapped to genome of interest	• good for low copy number – may be just a few molecules of miRNA/cell • no requirement for primer so "unbiased" technique – all RNA is captured • quantitative detection
realtime PCR	• RNA isolated and fragmented • cDNA synthesis (specialized primers required) • quantification against synthetic miRNA copies	• technically challenging – binding affinity of primers difficult to balance
Northern blot	• RNA extracted • RNA fractionated (electrophoresis) • probe detection	• large amounts of RNA required
in situ hybridisation	• whole mount preparations • probes applied directly to tissues	• precursor miRNA easier to detect (longer) • modified probes required for shorter sequences

Table 9.2. Summary of detection methods for miRNAs

MicroRNAs are of clinical interest in that the gene expression they regulate is increasingly recognized to be important in a range of diseases - cardiovascular (289), malignant (291, 292) and, more recent, immune disorders (293). Detection of miRNAs in plasma includes the detection of placental miRNAs in pregnancy (294). While this is evidently of interest in the context of antenatal diagnosis of fetal genetic disorders, the potential for investigation of intrinsic placental parenchymal disorders is real.

APPENDICES

APPENDIX A: LREC APPROVAL

Cambridgeshire 3 Research Ethics Committee
(formerly Peterborough & Fenland Research Ethics Committee)
Victoria House
Capital Park
FULBOURN
Cambridge
CB21 5XB

Telephone: 01223 597597
Facsimile: 01223 597645
05 June 2007

Dr Flora Jessop
Consultant Paediatric/Perinatal Pathologist
Department of Pathology, Box 235
Addenbrooke's Hospital
Hills Road
Cambridge
CB2 0QQ

Dear Dr Jessop

Full title of study: **Outcomes in pregnancy and the neonatal period: correlation with placental examinations**
REC reference number: **07/Q0106/51**

Thank you for your letter of 08 May 2007, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information was considered at the meeting of the Sub-Committee of the REC held on 04 June 2007. A list of the members who were present at the meeting is attached.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised. The favourable ethical opinion is conditional upon the revised

Consent Form being given an updated version number and/or date. Please send the Committee a copy of the revised Consent Form as soon as possible. Please note that the approved Patient Information Sheet is the revised sheet of 080507.

Ethical review of research sites

The Committee has designated this study as exempt from site-specific assessment (SSA. There is no requirement for [other] Local Research Ethics Committees to be informed or for site-specific assessment to be carried out at each site.

Conditions of approval

The favourable opinion is given provided that you comply with the conditions set out in the attached document. You are advised to study the conditions carefully.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
Application (Lock code: AB/106222/1)		19 March 2007
Investigator CV: Dr Flora Jessop		15 March 2007
Protocol	1.1	19 March 2007
Covering Letter: Re initial application		19 March 2007
Letter from Sponsor: Priya Shimoga, Addenbrooke's R&D		22 March 2007
Patient Information Sheet		08 May 2007
Participant Consent Form		
Participant Consent Form		
Response to Request for Further Information: Letter from Dr Flora Jessop		08 May 2007
Letter from funder: Keith Day		30 October 2006
Applicant's checklist		19 March 2007

R&D approval

All researchers and research collaborators who will be participating in the research at NHS sites should apply for R&D approval from the relevant care organisation, if they have not yet done so. R&D approval is required, whether or not the study is exempt from SSA. You should advise researchers and local collaborators accordingly.

Guidance on applying for R&D approval is available from http://www.rdforum.nhs.uk/rdform.htm.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

Feedback on the application process

Now that you have completed the application process you are invited to give your view of the service you received from the National Research Ethics Service. If you wish to make your views known please use the feedback form available on the NRES website at:

https://www.nresform.org.uk/AppForm/Modules/Feedback/EthicalReview.aspx

We value your views and comments and will use them to inform the operational process and further improve our service.

07/Q0106/51	**Please quote this number on all correspondence**

With the Committee's best wishes for the success of this project

Yours sincerely

Mr Stuart Kent
Vice-Chair

Email: lynda.mccormack@eoe.nhs.uk

Enclosures: *List of names and professions of members who were present at the meeting and those who submitted written comments*

Standard approval conditions

Copy to: *Dr Claudia Rizzini*
Research and Development Manager
R & D Department, Box 277
Addenbrooke's Hospital
Hills Road
Cambridge
CB2 0QQ

Cambridgeshire 3 Research Ethics Committee

Attendance at Sub-Committee of the REC meeting on 04 June 2007

Written comments received from:

Name	*Position (or reason for attending)*
Dr Doug Johnston	Lay member (retired consultant paediatrician)
Mr Stuart Kent	Retired consultant surgeon

In attendance:

Name	*Position (or reason for attending)*
Mrs Lynda McCormack	REC Assistant Administrator

APPENDIX B - PATIENT INFORMATION LEAFLET AND CONSENT FORM

Studies on your Placenta

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Talk to others if you wish. This leaflet:
Outlines the aim of the study and what will happen to you if you take part.
Outlines more detailed information about the conduct of the study.
Please do ask us if there is anything that is not clear or if you would like more information.
Take time to decide whether or not you wish to take part.

2. What is the purpose of the study?
This study focuses on the placental examination and is being carried out jointly between the Rosie Hospital, Addenbrooke's Histopathology Department & Great Ormond Street Hospital for Sick Children. We plan to examine placentas from women delivering their babies at the Rosie over a two year period, and will correlate the findings to pregnancy outcome and establish baseline features of the placenta.

3. Why have I been chosen?
We are asking all mothers who are expecting one baby (i.e. not twins) booking at the Rosie.

4. Do I have to take part?
No. It is up to you to decide whether or not to take part. If you do, you will be given this information sheet to keep and be asked to sign a consent form. Once you have signed the consent form, we will send your placenta for pathological examination and there is nothing further you would need to do.

5. What will happen to me if I take part?
We are asking only one thing: to be able to examine your placenta (afterbirth) once baby is born. It is normally disposed of, however we will examine it in the laboratory and link the details of your pregnancy outcome anonymously with your maternity and baby's records. This study will not in any way affect the care you receive, your appointments or delivery. You will not be contacted after the study is over.

6. What do I have to do?
You will be provided the relevant information of the study and then asked if you wish to take part in the study. You then need do is to sign the consent form (part 2) attached to this information sheet.

7. What are the possible benefits of taking part?
Normally, placentas are not sent for pathology examination. There is no direct benefit to you from taking part in the study, however as you are participating in this study, we'll examine the afterbirth and a report would be available for, if requested by your doctor in the hospital looking after you during the pregnancy.

8. What happens to my placenta?
Studies have linked the pregnancy and neonatal outcomes with the changes in the placenta. We'll examine the placentas and link the examination findings to the pregnancy and

neonatal outcome. The placenta (afterbirth) will be examined in the laboratory and some small pieces of tissue will be taken and examined under the microscope. The tissue will then be held in long term (minimum 25 years) secure storage at Addenbrooke's and Great Ormond Street Hospitals. These tissues from the placenta will be used for the further studies which will be subjected to ethical approval.

9. Will my taking part in the study be kept confidential?
We will follow ethical and legal practice and all information about you will be handled in confidence. Information will be collected by one of the research team members. The anonymised database linking placental pathology with all outcome data will be kept on a restricted research database at Addenbrooke's.

10. What if there is a problem?
If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions (S Pathak, ext: 58137). If you remain unhappy and wish to complain formally, you can do this through the NHS Complaints Procedure. Details can be obtained from the hospital.

11. What will happen to the result of the study?
We intend to use the result of this study in future pregnancies management. We intend to publish the results and you will not be identified in any report/publication.

12. Who has reviewed the study?
All research in the NHS is looked at by independent group of people, called a Research Ethics Committee to protect your safety, rights, wellbeing and dignity. This study has been reviewed and given favourable opinion by Peterborough Research Ethics Committee.

13. Who will be doing the research?
The research group is: Mr C Lees (Consultant in Obstetrics and Fetal Medicine), Mr G Hackett (Consultant Obstetrician and Gynaecologist), Dr E Murdoch (Consultant Neonatologist), Dr F Jessop (Consultant Paediatric Pathologist), Dr N Sebire (Consultant Pathologist), Dr L Hook (Pathology Subspeciality Fellow) and Dr S Pathak (Research Fellow in Fetal Medicine). The study funded jointly by Addenbrooke's Charities (Charity number: 1048868), Cambridge Fetal Care, and Great Ormond Street Hospital.

14. Contact Details:
The research fellow conducting this study is: Dr Sangeeta Pathak If you have any queries about this study, observations or complaints, then please contact her by telephone (extension 58137, or through switchboard via bleep) or email: sangeeta.pathak@Addenbrooke's.nhs.uk

Patient Identification Number for this trial:

CONSENT FORM
Title of Project: Placental Correlates of Pregnancy and Neonatal Outcomes.

Name of Researcher: Dr Sangeeta Pathak

1. I confirm that I have read and understand the information sheet dated…… for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily ☐.

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected ☐.

3. I understand that relevant sections of any of my medical notes, my baby's medical Notes and data collected during the study, may be looked at by responsible individuals from the research team of Cambridge University Hospitals NHS Trust and Great Ormond Street Hospital where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records ☐.

4. I consent for my placental tissues to be stored at the Addenbrooke's Hospital and Great Ormond Street Hospital for further studies ☐.

5. I agree to take part in the above study ☐ .

_____	_____	_____
Name of Patient	Date	Signature

_____	_____	_____
Name of Person Taking consent	Date	Signature

When completed, 1 for patient; 1 for researcher site file; 1(original) to be kept in medical notes.

APPENDIX C: HISTOPATHOLOGY REQUEST FORM

East Anglia Perinatal and Paediatric Histopathology Services and Fetal Medicine Department.

REQUEST FORM

(Study contact: Dr Sangeeta Pathak ext 3660.)

Request for Examination of Placenta for the **Studies on Your Placenta Project**
LREC Reference no. **07/Q0106/51** (A completed consent form must be filed in the notes).

Section 1

Addressograph

Gestation:

Delivery date:

Ensure that, if clinically required, samples for Cytogenetics + Microbiology have been taken.

If placental histopathology is required, complete green form as normal and enclose with this form.

Section 2:

Name of person completing this form:

Name of person taking consent:

Section 3
FOR RESEARCH USE ONLY

RNA later sample taken: Y N

(Page 2: for laboratory use only)

Page 2: please note, this page for laboratory use only

Department of Histopathology, Addenbrooke's Hospital Cambridge

PLACENTA WEIGHT SHEET

Number		**Name**	
Hospital number		**Date**	
Technician		**Path**	

MACRO

Trimmed	
Dimensions	
Cord length	
Cord diameter	
Number of vessels	
Insertion	
Direction of coil	
Coiling	

8		H	
9		J	
10		K	
11		L	
12		M	
13		N	

1		A	
2		B	
3		C	
4		D	
5		E	
6		F	
7		G	

MICRO

APPENDIX D – PROTOCOL FOR MACROSCOPIC EXAMINATION OF PLACENTAS

PROTOCOL FOR EXAMINATION OF PLACENTAS

Macroscopic examination:
Placentas will be received in the histopathology department, Addenbrooke's Hospital. The specimens should be fresh and placed in labelled clean plastic buckets with a labelled request form. Cord and membranes will be trimmed from the fresh specimens. Samples from both fetal and maternal surfaces will be taken and snap frozen in liquid nitrogen.

Checklist for cord examination:	length (cm) diameter (cm) coiling index (turns/length) direction of cord spiral cord insertion site true knots
Checklist for placental examination:	trimmed weight (g) measurements in 3 dimensions (cm) present/absence of: infarct (s), haematoma, fibrin, maternal surface thrombus
Standard blocks:	cord x 3 (maternal end, fetal end, central) membranes (in same cassette as one cord block) central parenchymal blocks (full-thickness) x 3 peripheral parenchymal block (full-thickness) x 1 maternal surface, 2 sections in 1 block any lesional tissue

Microscopic examination:

Checklist for reporting cord and membranes:
- funisitis
- chorioamnionitis
- maternal vascular disease in any adherent decidua
- meconium staining/necrosis
- amnion nodosum

Checklist for reporting placental parenchyma:
- chorionic plate
  - inflammation, infection, FTV
- stem villi
  - inflammation, infection, FTV, mesenchymal dysplasia, storage disease distal villi
  - hypoplasia, chorangiosis, VUE, infection, storage disease
- intervillous space
  - inflammation, infection, haemorrhage, fibrin (massive)
- maternal vessels
  - mural fibrin deposition
  - atherosis

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