THE EFFECT OF SEVERITY OF PRE-ECLAMPSIA ON THE BASAL PLATE INTERVILLOUS SURFACE LINING COMPONENTS-A CONFOCAL LASER SCANNING MICROSCOPY STUDY

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Bismarck Afedo Hottor BSc., MB ChB (Ghana)

Department of Infection, Immunity and Inflammation

University of Leicester

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Abstract

Using immunofluoresce microscopy and histology, intervillous surface lining components area fractions were measured in healthy term chorionic plate (CP) (n=7), healthy term placental basal plate (n=11), mild pre-eclamptic (MPE) placental basal plate (n=10) and severe pre-eclamptic (SPE) placental basal plate (n=11). The aims are (1) to examine the effect of pre-eclampsia (PE) and its severity on the basal plate (BP) intervillous surface lining composition and (2) to define the composition of the CP intervillous surface lining. The results show the mean birth weight of the babies in the SPE are significantly lower than in the HC and MPE (F (2, 29) = 11.912, p =0.000) and the mean gestational age at delivery (GAD) in the SPE group is significantly lower than the HC and MPE (GAD) [Anova p = 0.001; posthoc, p =0.003 and p = 0.001 for MPE and healthy control (HC) respectively]. The mean anchoring villi (AV) to fibrin (NS) ratio of the BP intervillous surface lining is significantly lower in the SPE compared to MPE and the HC groups (ANOVA, p = 0.010; posthoc p = 0.009 and p = 0.628 for HC and MPE respectively) indicating increase fibrin deposition on the basal plate and decreasing anchoring villi attachment area with increasing severity of pre-eclampsia. There are no significant differences in the mean length fractions of endothelium and trophoblast between the 3 groups (Anova, p= 0.107 and p = 0.131 respectively). CP intervillous surface is lined partly by endothelium, trophoblast and an acellular material (fibrin/fibrinoid). Conclusions: (1) The inverse relationship between the proportions of fibrin and anchoring villi with increasing severity of pre-eclampsia indicates that increase deposition of the BP fibrin and poor trophoblast invasion of the BP underlies the disease progression. (2) The CP is partly endothelial contrary to the widely accepted view of wholly trophoblast.

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PUBLICATIONS BASED ON WORK PRESENTED IN THIS THESIS

Full Papers

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Variation in composition of the lining of the intervillus space in term placentas of mothers affected by mild to severe pre-eclampsia. *American Journal of Obstetrics and Gynecology,* (Awaiting submission)

Ahenkorah, J., Hottor, B., Byrne, S., Bosio, P., Ockleford, C. D., 2009. Immunofluorescence confocal laser scanning microscopy and immuno-electron microscopic identification of keratins in human materno-foetal interaction zone. *Journal of Cellular and Molecular Medicine,* **13**, 735-748.

Byrne, S., Ahenkorah, J., Hottor, B., Lockwood, C., Ockleford, C. D., 2007. Immuno-electron microscopic localisation of caveolin 1 in human placenta. *Immunobiology,* **212**, 39-46.

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Ahenkorah, J., Hottor, B., Davis, D., Byrne, S., Waugh, J., Bosio, P., Ockleford, C. D., (2007**)**

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For the Late Torgbe Gborwonu V, known in private life as Mr Stephen Hottor Dzekle, Elinam, Klenam and Patricia.

CONTENTS

LIST OF ABBREVIATIONS

ACE	Angiotensin converting enzymes
ACOG	American College of Obstetrician and Gynecologists
ALT	Alanine transaminases
AST	Aspartate transaminases
AV	Anchoring villi
BP	Blood pressure
BP	Basal plate
BPP	Biophysical profile
CD	Cluster of differentiation molecule
CG-H	Hyperglycosylated human chorionic gonadotropin
CLSM	Confocal Laser scanning microscope
CNS	Central nervous system
CP	chorionic plate
CRH	Corticotrophin releasing hormone
CSF-1	Colony stimulating factor-1
CV	Chorionic villous
DABCO	Diazabicyclone [2-2-2] octane
DARC	Duffy antigen receptor chemokines
DBP	Diastolic blood pressure
df	degree of differentiation
DIC	Disseminated intravascular coagulation
DNA	Deoxyribonucleic acid
DOB	Date of birth
ECM	Extracellular matrix molecules

EDD	Expected date of delivery
EGF-R	Epidermal growth factor receptor
EMBIC	European Network of Excellence on Embryo Implantation Control
END	Endothelium
EPCR	Endothelial protein C receptor
EVT	extravillous trophoblast
FGR	Fatal growth retardation
FHPE	Family history of pre-eclampsia
FITC	Fluorescein isothiocyanate
GAD	Gestational age at delivery
GFR	Glomerular filtration rate
HC	Healthy control
HDBP	Highest diastolic blood pressure
HELLP	Haemolysis, Elevated Liver enzymes, Low platelet count
HLA	Human leukocyte antigens
HSBP	Highest systolic blood pressure
I-CAM	Intercellular cell adhesion molecule
IGF	Insulin-like growth factor
IUGR	Intrauterine growth retardation
IVS	Intervillous space
K	Keratin/cytokeratin
KIR	Killer Immunoglobin-like Receptor
K-S	Kolmogorov-Smirnov test
LDH	Lactate dehydrogenase
LFT	Liver function test
LRI	Leicester Royal Infirmary
MHC	Major Histocompatibility complex

MMP	Matrix Metalloproteinases
MPE	mild pre-eclampsia
MRC	Medical Research Council
mRNA	messenger Ribonucleic Acid
MSB	Martius Yellow-Brilliant Crystal Scarlet Blue
N-CAM	Neutral cell adhesion molecule
NHS	National Health Service
NK	Natural killer cells
NS	Non-specifically labelled (Fibrin)
NST	Non-stress test
PAI	Plasminogen activator inhibitors
PAL-E	Pathologische anatomie Leiden-endothelium
PCNA	Proliferating cell nuclear antigen
PECAM	Platelet-Endothelial cell adhesion molecule
PIGF	Placental growth factor
PL	Placental lactogen
PMH	Past medical history
PROM	Premature rupture of membrane
RBC	Red blood cell
SBP	Systolic blood pressure
sFlt-1	Soluble fms-like tyrosine kinase 1
SLE	Systemic lupus erythematosis
SPE	Severe pre-eclampsia
sVEGFR	Soluble vascular endothelial growth factor receptor
S-W	Shapiro-Wilk’s test
TBS-T	Tris buffered saline containing Tween 20
TF	Tissue factor

TFPI-1	Tissue factor pathway inhibitor-1
TFPI-2	Tissue factor pathway inhibitor-2
TIMP	Tissue Inhibitors of metalloproteinases
TM	Thrombomodulin
TNF	Tumor necrosis factor
tPA	Tissue type plasminogen activator
TPB	Trophoblast
UEA-1	Ulex europaeus agglutinin
UGMS	University of Ghana Medical School
uPA	Urokinase type plasminogen activator
VEGF	Vascular endothelial cell growth factor
vWF	von Will brand factor
WHO	World Health Organization
β-hcG	Beta human chorionic gonadotropin

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1 INTRODUCTION

Gross Features of Human placenta

The placenta is a transitory organ formed from fetal and maternal cells and acts as the medium of exchange between the fetus and the mother in most viviparous vertebrates, including all true mammals during pregnancy (Boyd and Hamilton, 1970).

The shape of a delivered human term placenta is discoid, flat round to oval in more than 90% of cases (fig 1.1 and 1.2). In the remaining 10% of cases, various shapes such as bilobata, placenta duplex, placenta succenturiata, placenta zonaria, and placenta membranacea have been described (Benirschke and Kaufman 2000).

The average weight of term placenta is 470g whilst its average diameter and thickness are 22cm and 2.5cm respectively (Benirschke and Kaufman 2000). These parameters depend on the mode of delivery, the timing of cord clamping and the time lapse between delivery of the placenta and examination (Benirschke and Kaufman 2000).

The human placenta is classified as deciduate, villous and haemo-chorial (Boyd and Hamilton, 1970). It is described as deciduate because at parturition part of maternal endometrial tissues are sheared with the organ. The placental chorionic villi greatly increase the fetal surface exposed to maternal blood and thus improve exchange of materials between mother and the baby. Grosser described human placenta as haemo-chorial meaning that fetal tissues, in this case trophoblast, are in intimate contact with maternal blood (Boyd and Hamilton, 1970).

The placenta has two surfaces; the foetal (or chorionic or amniotic) surface (fig. 1.1) and maternal (or uterine) surface (fig.1.2).

Fetal surface of the placenta

The chorionic surface is formed by the chorionic plate with branches of fetal blood vessels (Boyd and Hamilton, 1970). It looks glossy and largely transparent because it is covered by the intact epithelium of the amnion. The umbilical cord is inserted into this surface. The umbilical cord is inserted eccentrically in between 54 and 92% of cases (Boyd and Hamilton, 1970). Other points of umbilical cord insertion include central, marginal and velamentous. When the cord inserts into a membrane on the margin of the placenta the phenomenon is called battledore placenta.

Branches of the umbilical vessels are clearly visible on this surface as they radiate from the point of umbilical cord insertion outwards. At the arteriovenous crossings (fig. 1.1), the arteries usually cross the veins on their amniotic aspect (Benirschke and Kaufman 2000). The reverse is true only in 3% of cases (Wentworth, 1965). The areas in between the chorionic vessels are transparent and it is a purplish blue to black colour which is produced by maternal blood in the blood space of the placenta. The chorionic plate sometimes has opaque spots (bosselations) or opaque areas. These are due to subchorial deposition of Langhan's fibrinoid. A ring of opaque tissue is present near the placental margin, at the point where the chorionic vessels are absent. This ring forms a boundary between the central concave part of the fetal surface where chorionic vessels are visible and the margin of the placenta proper and it is called the subchorial closing ring (Boyd and Hamilton, 1970). The origin of the cells in this ring is debatable. Some people believe the cells as decidual but others (Ortmann, 1955, Boyd and Hamilton, 1970, Benirschke and Kaufman 2000) thought that the ring was formed as a result of the presence of increased cytotrophoblast cells and collagen fibres. The placental membranes insert into the placenta here. Occasionally this ring is broad and prominent and in such situations the placenta is referred to as placental marginata. When the ring has villous tree undergrowth at its peripheral margin, placenta circumvallata is said to be formed (Benirschke and Kaufman 2000).

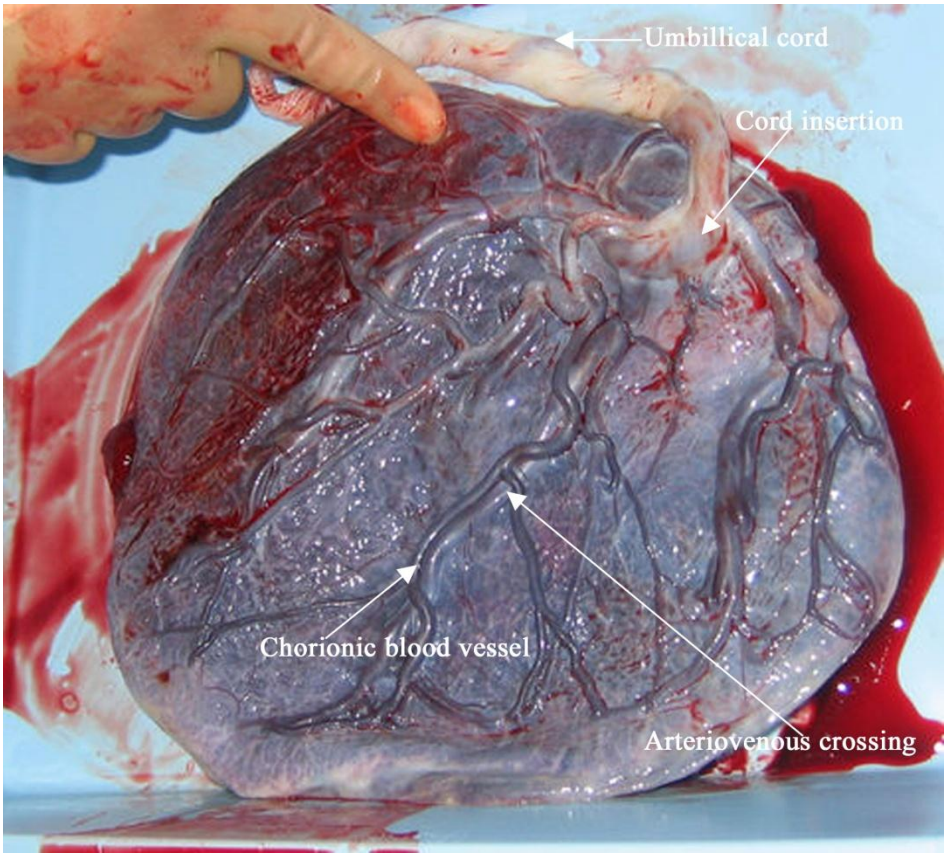


Fig. 1.1 The chorionic surface of a freshly delivered human placenta modified Photo by Jeremy Kemp, 4/9/05*:* http://commons.wikimedia.org/Image;Human_placenta_baby_side

Maternal surface of the placenta

Maternal surface (Fig1.2) is opaque in the delivered placenta as it is formed from the laminar degenerative processes within the junctional zone that result in placental separation from the uterus during the third stage of labour (Benirschke and Kaufman 2000). It is incompletely divided by a system of grooves into 10-40 lobes also called maternal cotyledons (Boyd and Hamilton, 1970, Benirschke and Kaufman 2000). These grooves and the corresponding internal tissues that partially divide the intervillous space were named placental septae by William Hunter in 1774 (Benirschke and Kaufman 2000).

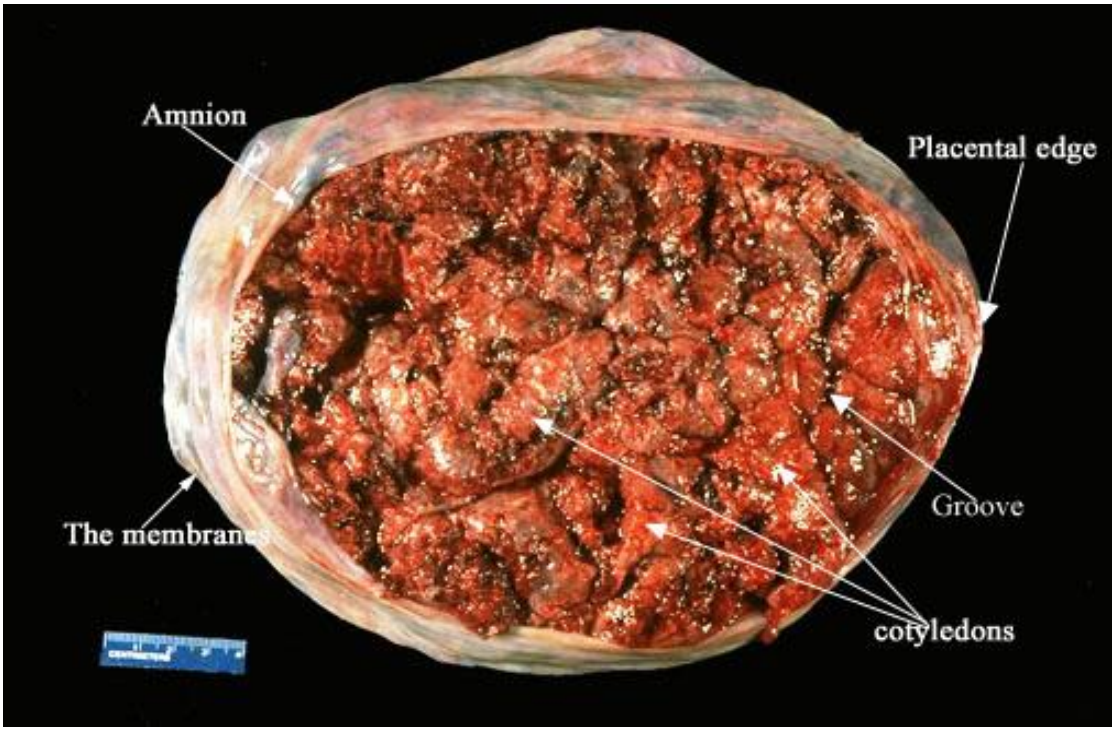


Fig. 1.2: The maternal surface of Human placenta showing the grooves that divide the surface into maternal cotyledons. The amnion sac is retracted to the edge of the placenta.

Each of these incomplete compartments (maternal cotyledon) corresponds fairly well with the position of a villous tree (foetal cotyledon) (Benirschke and Kaufman 2000), but rarely does each one contain a single villous tree (Boyd and Hamilton, 1970). About 60 to 70 stem villi arise from the chorionic plate and each branches into a villous tree. Each lobe therefore, contains one or more villous trees. Some small lobes contain only one stem villous and these are described as placentone (Benirschke and Kaufman 2000).

The intervillous space is a diffuse space that is sandwiched between the chorionic plate and the basal plate. It is derived from expansion and coalescence of the lacunae system. The intervillous space contains chorionic villous trees, cell islands, septa, fibrinoid deposits and maternal blood (Boyd and Hamilton, 1970, Benirschke and Kaufman 2000).

Placental circulation

Two different circulatory systems, maternal and foetal, come together for the purpose of nutrition of the foetus even though they do not mix. Maternal blood is pumped into the intervillous space through the spiral arteries and the space is drained by uteroplacental veins. These arteries are plugged during early stages of placental development (Hustin *et al.*, 1988, Burton *et al.*, 1999). They recannalize at approximately 12 weeks post-conception (Jauniaux *et al.*, 2000). There is therefore little blood in the intervillous space before then. The spiral arteries are remodelled by endovascular and interstitial extravillous cytotrophoblast cells and they lose their smooth muscle cells and become wide bore vessels with low resistance (Brosens *et al.*, 1967, De Wolf *et al.*, 1973, Pijnenborg, 1996, Zhou *et al.*, 1997). These vessels deliver large amount of blood at low pressure into the intervillous space. Incomplete transformation of these vessels is now known to underlie pregnancy complications such as pre-eclampsia and intrauterine growth retardation (Robertson *et al.*, 1967, Brosens *et al.*, 1972, Brosens *et al.*, 1977, Khong *et al.*, 1986, Pijnenborg *et al.*, 1991).

The umbilical cord has two arteries and one vein (Boyd and Hamilton, 1970). These vessels branch at the point of the cord insertion into chorionic vessels. They divide and enter the stem villi. In the villi, they branch to form a network known as the foetal arteriocapillary-venous system. Deoxygenated blood from the foetal heart is pumped via the umbilical arteries into the villi and here they are oxygenated by diffusion of oxygen from the maternal blood that is in the intervillous space (Moore *et al.*, 2008). The diffusion barrier (materno-foetal barrier) consists of syncytiotrophoblast, cytotrophoblast and its basal lamina and foetal capillary endothelium (Boyd and Hamilton, 1970). The oxygenated blood is then carried by the umbilical vein back to the tissues of the baby. The highly branched villi are the main site of exchange between foetus and the maternal circulation

Fibrin deposition in the placenta

Fibrin deposition is a common feature in human placenta. In pregnancy, fibrin is deposited in the placenta from 12-13 weeks of gestation till term (Boyd and Hamilton, 1970, Kaufmann *et al.*, 1996a). It is seen in the intervillous space, mainly the villous surface (Fox, 1967, Fox, 1975, Kaufmann *et al.*, 1996a, Demir *et al.*, 1997, Mayhew *et al.*, 2000), basal plate intervillous surface and within the basal plate (Frank *et al.*, 1994, Lang *et al.*, 1994, Kaufmann *et al.*, 1996b, Mayhew *et al.*, 2000). Fibrin-type fibrinoid is deposited mainly at places where there is syncytiotrophoblast loss (Nelson *et al.*, 1990, Nelson, 1996).

Fibrinoid of the intervillous space including the intervillous surface of the basal plate is described as fibrin-type fibrinoid (Frank *et al.*, 1994, Lang *et al.*, 1994). It is essentially a blood clot product of maternal blood in the intervillous space (Frank *et al.*, 1994, Lang *et al.*, 1994). The amount of fibrin-type fibrinoid deposited in the intervillous space is therefore determined by the balance between the coagulation and the fibrinolytic systems (Roberts and Schwartz, 2002). In normal pregnancy the balance it tipped in favour of coagulation especially towards term (Shaper *et al.*, 1965, Oliver *et al.*, 1976, Fletcher *et al.*, 1979). This is an adaptation to cope with haemorrhage associated with parturition.

Blood Clotting in the placenta is regulated by protein C or S of maternal origin and tissue factor inhibitors, thrombomodulin, endothelial protein C receptor (EPCR) and annexin V, of foetal origin (Lanir *et al.*, 2003). Other regulators are from both maternal and foetal sources e.g. tissue factor pathway inhibitor (Lanir *et al.*, 2003). The fibrinolytic system is also regulated by tissue-type plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA).

The volume of fibrin in the intervillous space and the volume of intervillous space both increase towards term (Aherne and Dunnill, 1966, Boyd, 1984). Similarly, the volume of intervillous space fibrinoid, intervillous space volume and the surface area of the villi

correlates positively (Mayhew and Barker, 2001). This will suggest that in normal pregnancy, the volume of the intervillous space fibrin reflects the state of the intervillous space perfusion (Fox, 1967, Fox, 1975). There are contrasting views about the volume of intervillous space fibrinoid in abnormal pregnancies (Aherne and Dunnill, 1966, Teasdale, 1985, Teasdale, 1987, Kaufmann *et al.*, 1996b, Mayhew *et al.*, 2000).

Functions of the placenta

1. Transport of gases and nutrients. Oxygen is transferred from maternal to foetal blood and carbon dioxide moves in the opposite direction. Nutrients e.g. water, inorganic salts, vitamins, fats, proteins and carbohydrates in the mother's blood are taken up into the foetal blood. Foetal metabolic waste diffuses into maternal blood (Matsumura and England, 1992).

2. Metabolism- Placenta synthesises glycogen, cholesterol and fatty acids, particularly during early pregnancy and these serve as sources of nutrient and energy for the embryo/foetus (Moore *et al.*, 2008).

3. Endocrine hormone secretion-The placenta secretes hormones such as progesterone (which is important for maintaining pregnancy), somatomammotropin (human placental lactogen) (important for increasing blood levels of glucose and lipid in the maternal blood), oestrogen, relaxin, beta human chorionic gonadotropin(β-*hcG*), and insulin like growth factor (*IGF*) (Matsumura and England, 1992, Moore *et al.*, 2008).

4. Immuno-protection- Placenta secretes Neurokinin B containing the phosphocholin molecule. This helps the foetus to escape detection by maternal immune cells. Placenta also prevents some bacteria from crossing the placental barrier to the foetus (Matsumura and England, 1992).

Placental Basal Plate

The basal plate (BP) is defined as the maternal aspect of the intervillous space (Boyd and Hamilton, 1970). In a delivered placenta it is found on the surface opposite the insertion of the umbilical cord and is said to form the base of the intervillous space. The BP is a zone of the placenta where both maternal and foetal tissues come into close contact and interact intimately with each other (Benirschke and Kaufman 2000).

The BP of mature term placenta is a complex structure, composed of tissues such as extravillous trophoblast, endometrial stroma, fibrinoid, remnants of degenerating villi, and utero-placental vessels (Benirschke and Kaufman 2000).

Development of the Basal Plate

The primordial structure which gives rise to the basal plate is called the trophoblastic shell. This is a layer of trophoblast cells that separates the lacunal system of the implanting blastocyst from the endometrial tissues. The trophoblastic shell in the initial stages of pregnancy is made up, more or less, of syncytiotrophoblast. It represents the front of the invading embryo. By day 13 post conception (Benirschke and Kaufman 2000), migrating cytotrophoblast cells, through trabeculae, eventually reach and penetrate the shell. These cells now gain direct contact with the endometrial tissue. They then transform into invading cells called extravillous trophoblast cells by losing their compact epithelial nature. The exact border between the trophoblastic shell and the endometrium is lost and the term basal plate replaces trophoblastic shell. This stage is documented as 22 days post-conception in humans (Larsen and Knoth, 1971)

The BP therefore includes the base of the intervillous space and all maternal and foetal tissues that are attached to it after delivery of the placenta. When the placenta is in-situ, the BP and the placental bed constitute the junctional zone and the two cannot be identified separately (Benirschke and Kaufman 2000). Fibrinoid in the BP is formed from necrotic degeneration of decidua and trophoblast with accumulation of fibrin. Extravillous trophoblast cells secrete fibrinoid of the placenta (Frank *et al.*, 1994, Frank *et al.*, 1995).

Layers of the definitive basal plate

The definitive BP is between 100µm-1.5mm thick (Benirschke and Kaufman 2000). Its layering varies from place to place and only rarely are the developmental layers preserved (Hein, 1971). The various tissues making up the basal plate are illustrated in fig. 1.3 below.

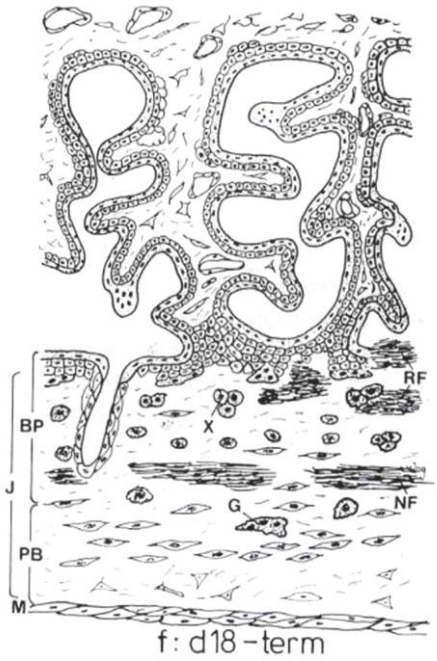


Fig. 1.3: A drawing of the transverse section through mature term placenta, J = junctional zone, BP = basal plate, PB = placental bed, X = Extravillous cytotrophoblast cells, RF = Rohr's fribrinoid, NF = Nitabuch's fribrinoid, G = Trophoblast giant cells, M = myometrium. (Diagram from Pathology of the Placenta, 2000).

The Intervillous Surface lining of the Basal Plate

The intervillous surface of the BP is lined by a mosaic consisting of a unicellular layer of trophoblast, a unicellular layer of endothelium and fibrin/fibrinoid (Byrne *et al.*, 1998, Byrne *et al.*, 2001, Smith *et al.*, 2004, Richani *et al.*, 2007). Previously this was thought to be lined entirely by fibrin or fibrinoid and trophoblast (Boyd and Hamilton, 1970). The endothelium at this site is of maternal origin (Byrne *et al.*, 2001, Richani *et al.*, 2007) and is thought to originate from the openings of maternal uteroplacental vessel (Smith *et al.*, 2004) or from endothelial progenitor cells present in the maternal circulation (Richani *et al.*, 2007). It is a unique layer since endothelium, of mesodermal origin, makes contact with, and probably shares junctional organelles with trophoblast of epidermal origin. The cellular lining is at some places interrupted and replaced by anchoring villi and fibrin or fibrinoid (Smith *et al.*, 2004). The endothelium occupies a larger proportion of the intervillous border of the basal plate compared to trophoblast (Smith *et al.*, 2004, Richani *et al.*, 2007).

The Rohr's stria

This stria was described in great detail by Rohr in 1889 (Benirschke and Kaufman 2000). It forms part of placental fibrinoid. Grosser in 1925 defined fibrinoid of the placenta as all placental material of solid consistency that is not composed of cells, syncytium, or connective tissue that show special affinity to acid stains (Kaufmann *et al.*, 1996b).

The Rohr's stria is patchy and rather focal in its distribution. It replaces the cellular lining of the intervillous surface of the basal plate at places where the cells are absent (Smith *et al.*, 2004, Richani *et al.*, 2007). This fibrinoid is derived from the polymerization of fibrinogen in the blood (Sutcliffe *et al.*, 1982) and as a result, it reacts with anti -fibrinogen antibodies (Moe, 1969). Because of its intervillous location, it is largely formed from maternal blood that bathes the intervillous space although it is partly from fetal blood (Frank *et al.*, 1994).

The superficial fibrinoid layer of the basal plate is said to be largely fibrin-like in its biochemical composition (Frank *et al.*, 1994) and it shows intense reactivity with antifibrin antibodies which have no cross-reactivity with fibrinogen (Hui *et al.*, 1983). It contains plasma fibronectins but it is devoid of basal lamina molecules and interstitial matrix molecules such as cellular fibronectins, collagen and laminin (Frank *et al.*, 1994). This type of fibrinoid binds to lectin, indicating the presence of degenerating remnants of endothelium and blood cells (Lang *et al.*, 1994). There are no extravillous trophoblast cells embedded in it and ultrastructurally, it exhibits a dense meshwork of fibres measuring less than 10nm in thickness, with characteristic cross-striations of fibrin filaments and an approximately 20nm periodicity (Frank *et al.*, 1994)

The functions of Rohr's fibrinoid includes regulating intervillous blood flow (Stark and Kaufman 1974) by obstructing all poorly perfused areas of the placenta during the establishment of the maternal circulation in the placenta. It is also thought to function as a barrier to trophoblast invasion since it is never invaded by trophoblast cells (Frank *et al.*, 1994). Another suggested function is as an immunological barrier that inhibits foetal antigens from coming into contact with maternal antibodies (Bardawil and Toy, 1959, Kirby *et al.*, 1964, Currie and Bagshawe, 1967, McCormick *et al.*, 1971, Azab *et al.*, 1972). This function is similar to the barrier function of fibrin components of tumours (Dvorak *et al.*, 1983)

Connective Tissue Layer

This layer forms the main part of the basal plate and is between 50µ to 1mm thick (Benirschke and Kaufman 2000). Tissues present in this layer include EVTs, endometrial stroma cells, connective tissue, decidual cells, remnants of anchoring villi and cell columns

(Benirschke and Kaufman 2000). It also contains a number of multinucleated giant cells (Norwitz *et al.*, 2001)

Extravillous Trophoblast Cells (EVT cells)

Extravillous trophoblast cells represent all syncytial and cellular trophoblast outside the villi (Kaufmann and Castellucci, 1997). The EVTs develop from the ectodermal layer of the blastocyst and so are epithelial cells but they lack a recognised basal lamina. The EVTs reach the basal plate through cell columns that connect the basal plate to anchoring villi.

Two forms of the EVTs are present in the basal plate: proliferative and invasive phenotypes (Kaufmann and Castellucci, 1997). The later phenotype predominates. The proliferative EVT cells are located in the proximal cells zone of the cell columns and in the first and second trimester placentae they express proliferation markers such as Ki-67, MIB-1, and anti-PCNA antibodies or incorporate H^3-thymidine (Bulmer *et al.*, 1988, Castellucci *et al.*, 1993, Blankenship and King, 1994a). The invasive type of EVTs is located in the deeper part of the basal plate i.e up to the distal third of the myometrium, and they differentiate from the proliferating cells (Bulmer *et al.*, 1988).

There are two types of invasive EVTs based on their location; endovascular and interstitial (Kaufmann *et al.*, 2003). The former invades uteroplacental vessels. Two types of endovascular EVTs are known; intramural EVTs which invade the walls of uteroplacental vessels, and intraarterial which replaces endothelium of vessels as well as form intraluminal plugs. The interstitial EVT cells derived from the cell column are in close contact with decidual cells, maternal immune cells like N-K cells and macrophages in the depth of the basal plate. The EVTs are embedded in matrix type fibrinoid (Frank *et al.*, 1994).

They express various surface molecules that characterises the level of differentiation, functions and roles in successful implantation. These factors include;

I) Growth factor receptors and their ligands: The pattern of expression of growth factor receptors and their ligands, and protooncogene products by EVTs support the interpretation that proliferation, differentiation and invasion are characteristic of these cells. Epidermal growth factor receptor (EGF-R) encoded by the protooncogene c- erbB-1, for example, is expressed on the proximal EVTs of the cell column that also express proliferation markers (Muhlhauser *et al.*, 1993, Duello *et al.*, 1994, Jokhi *et al.*, 1994). EGF is a potent stimulator of epithelial cell division. Maternal ligands that interact with EGF-R may play an important role in increasing the invasiveness of cytotrophoblast cells especially in early stages of placentation (Bass *et al.*, 1994). EGF was localised immunohistochemically in cytotrophoblasts and syncytiotrophoblasts providing evidence for its paracrine/autocrine role in trophoblast function (Hofmann *et al.*, 1992). The receptor encoded by protooncogene, c-erbB-2 on the other hand is present on all extravillous trophoblast cells that are negative for EGF-R (Muhlhauser *et al.*, 1993, Jokhi *et al.*, 1994). The gene product for the receptor for colony stimulating factor-1 (CSF-1), another mitogen, has also been localised on trophoblast cells. It is most intensely expressed on the early invasive trophoblast but weaker on the deeply invasive ones (Jokhi *et al.*, 1993) suggesting the presence of a differentiation gradient. C-kit ligand (stem cell factor), which is a blood cell growth factor, is expressed by invasive extravillous trophoblast cells throughout pregnancy (Sharkey *et al.*, 1994).

II) Expression of extracellular matrix receptors: Extravillous trophoblast cells express extracellular matrix (ECM) receptors, known as integrins. Differentiation of EVTs along the invasive pathway is accompanied by integrin switching so that different phenotypic trophoblasts express different integrins (Damsky *et al.*, 1992). Extravillous trophoblast stem cells i.e. the proliferating trophoblast that are attached to the basal lamina in the proximal cell column express α6β4 integrin (Damsky *et al.*, 1992, Aplin, 1993, Burrows

et al., 1993), a receptor for basement membrane constituent while those in the intermediate zone of the cell column express α5β1 fibronectin receptor. The invasive trophoblasts in the uterine wall are associated with upregulation of α1β1 collagen/ laminin receptor and α5β1 integrin. This integrin switching is said to regulate normal trophoblast invasion (Damsky *et al.*, 1994). In pre-eclampsia where there is shallow invasion of the uterus by trophoblast, abnormal integrin switching has been invoked as a possible mechanism (Zhou *et al.*, 1993).

III) Expression of cell-cell adhesion molecules: Trophoblast cells of the basal plate express cell-cell adhesion molecules such as E-Cadherin (Babawale *et al.*, 1995, MacCalman *et al.*, 1995), the neutral cell adhesion molecule (N-CAM) (Burrows *et al.*, 1994, King and Blankenship, 1995) and connexin 40 (Hellmann *et al.*, 1996, Winterhager *et al.*, 2000, Cronier *et al.*, 2002). E-cadherin is prominently expressed in the proximal proliferating cells and the intravascular trophoblasts but is absent in the invasive trophoblast cells in the intermediate zone (MacCalman *et al.*, 1995). N-CAM is expressed by all extravillous trophoblast cells in early placenta except the intramural trophoblast but in term placentae, it is restricted to the proximal proliferating trophoblast cells (Burrows *et al.*, 1994, King and Blankenship, 1995). N-CAM is responsible for cell-cell as well as cell-matrix adhesion. Other cell surface molecules expressed by trophoblast cells include carbohydrate sialyl-Lewis (King and Loke, 1988) and intercellular adhesion molecule, I-CAM found on the interstitial trophoblasts around the utero-placental vessels (Burrows *et al.*, 1994). These adhesion and gap junction molecules are important in regulating trophoblast invasion of the endometrium for successful placentation to provide the foetus with the high level of nutrition required for healthy development. EGF is known to regulate trophoblast invasion through modulation of connexin 40 (Wright *et al.*, 2006).

IV) Secretion of extracellular matrix: The extracellular matrix (ECM) in the placenta is called fibrinoid (Kaufmann and Castellucci, 1997). There are two types of fibrinoid based on ultrastructurally and immunohistochemical studies; the fibrin-type and matrix-type (Frank *et al.*, 1994, Lang *et al.*, 1994, Frank *et al.*, 1995). The matrix type fibrinoid is the ECM secreted by the invasive/interstitial trophoblast (Frank *et al.*, 1994, Frank *et al.*, 1995) and they are embedded in it. The matrix type fibrinoid is composed of various ECM molecules (Feinberg *et al.*, 1991, Castellucci *et al.*, 1993, Frank *et al.*, 1994, Huppertz *et al.*, 1996). Some of these component molecules include laminin, collagen IV, Heparan sulphate, fibronectins, vitronectin and blood group precursor antigen (Damsky *et al.*, 1992, Castellucci *et al.*, 1993, Frank *et al.*, 1994). Using immunohistochemistry and ultrastructural studies, the ECM is divided into three mosaic-like patches or compartments (Huppertz *et al.*, 1996)

a) Basal lamina-like extracellular matrix that contains laminin and collagen IV.

b) An amorphous, glossy substance that contain Heparan Sulphate and vitronectin

a) A fine fibrillar compartment with reactivity to fibronectin isoforms and oncofoetal isoforms (Huppertz *et al.*, 1996).

V) Secretion of proteinases, proteinase activators and inhibitors: In invading the endometrium, extravillous trophoblasts degrade ECM of the endometrium (Bischof and Martelli, 1992, Graham and Lala, 1992). Proteinases, their activators and inhibitors have been suggested in numerous studies to be important in this process (Fisher *et al.*, 1989, Emonard *et al.*, 1990, Moll and Lane, 1990, Autio-Harmainen *et al.*, 1992, Fernandez *et al.*, 1992, Blankenship and King, 1994b, Huppertz *et al.*, 1998a, Demir-Weusten *et al.*, 2007). The most studied proteinases are the Matrix metalloproteinases (***MMP)***. They are secreted by trophoblast in vitro (Bischof *et al.*, 1991) and their immunoreactivity has been

demonstrated in extravillous trophoblast cells and their surrounding extracellular matrix (Emonard *et al.*, 1990, Moll and Lane, 1990, Autio-Harmainen *et al.*, 1992, Fernandez *et al.*, 1992, Blankenship and King, 1994b, Huppertz *et al.*, 1998b, Demir-Weusten *et al.*, 2007). Matrix metalloproteinases 1, 2, 3, 9, 11 and membrane type ***MMP***s are immunoreactive in the extravillous trophoblast cells and decidual cells at the cell column and the basal plate as well as in in-vitro studies (Moll and Lane, 1990, Autio-Harmainen *et al.*, 1992, Fernandez *et al.*, 1992, Blankenship and King, 1994b, Polette *et al.*, 1994). The distribution pattern depends on the type of ***MMP.*** Antibodies against some of these proteinases are known to inhibit trophoblast invasion (Librach *et al.*, 1991) and their level of expression also decreases with increasing gestational age (Fisher *et al.*, 1989, Librach *et al.*, 1991, Polette *et al.*, 1994). ***MMPs*** are also thought to play a role in placenta separation as the level of the mRNA of ***MMP3*** and ***MMP9*** are raised during labour compared to post delivery (Bryant-Greenwood and Yamamoto, 1995). Recently, the enzymatic activity of ***MMP*** 3 and ***MMP*** 9 have been found to be highest at the contact region of the foetal and maternal parts, also suggesting their role in spontaneous separation of the placenta from the uterus during labour (Demir-Weusten *et al.*, 2007). The regulator of ***MMP*** activity is by their inhibitors, ***TIMP***s. So far ***TIMP***1 and***TIMP***2 have been described in human placenta, secreted mostly by decidual cells (Polette *et al.*, 1994) and to some extent by trophoblast cells (Ruck *et al.*, 1996, Huppertz *et al.*, 1998b). ***TIMP1*** and ***TIMP2*** have been demonstrated to completely inhibit human cytotrophoblast invasion in vitro (Librach *et al.*, 1991).

VI) Plasminogen activators expression: Plasminogen activators activate plasminogen to plasmin which is involved in degradation of extracellular matrix. Two types are known; urokinase-type (uPA) and tissue-type (tPA). uPA has been immunohistochemically localised on extravillous trophoblast cells particularly the differentiated and the invasive

type (Hofmann *et al.*, 1994). Other in-vitro evidence of expression of uPA by trophoblast has been provided by Bischof and Martelli (1992) and Eldar-Geva et al.(1993). Inhibitors of uPA and tPA have been found in human trophoblast (Astedt *et al.*, 1986, Feinberg *et al.*, 1989). The cytokine TNFα is found to inhibit trophoblast invasion mainly by increasing the expression of plasminogen activator inhibitors 1 (PAI-1) (Huber *et al.*, 2006).

VII) Expression of blood group precursor antigen: Oncofoetal carbohydrate epitope of the blood group precursor antigen i was localised immunohistochemically on invasive extravillous trophoblast cells (Frank *et al.*, 1995). This is i-glycosylated oncofoetal fibronectin-like molecule of about 55kDa which could be used as immunological cell surface markers for the invasive extravillous trophoblast (Frank *et al.*, 1995).

VIII) Hormone secretion: Human chorionic gonadotropin, hCG, is produced in pregnancy by trophoblast cells. Two forms of the hormone exist; regular hCG and its hyperglycosylated variant, CG-H (Cole, 1987, Elliott *et al.*, 1997). Extravillous trophoblast cells secrete CG-H whereas syncytiotrophoblasts secretes hCG (Lei *et al.*, 1999, Kovalevskaya *et al.*, 2002b, Cole *et al.*, 2006a, Handschuh *et al.*, 2007a). In the third completed week of gestation, CG-H accounts for 92% of the total hCG but its level declines with advancing gestation (Cole *et al.*, 2003, Sutton-Riley *et al.*, 2006). CG-H act in an autocrine manner to promote and modulate trophoblast invasion, tumour formation and placental growth (Lei *et al.*, 1999, Hamada *et al.*, 2005, Cole *et al.*, 2006a, Cole *et al.*, 2006b, Handschuh *et al.*, 2007b). The regular hCG that is produced by the villous syncytiotrophoblast cells on the other hand, promotes uterine vascularisation through uterine LH/hCG receptors (Lei *et al.*, 1999, Hamada *et al.*, 2005, Cole *et al.*, 2006a, Cole *et al.*, 2006b, Handschuh *et al.*, 2007b). Together, both hCG and CG-H are important in good placentation and therefore normal pregnancy (Cole, 2008). The gene for hCG and

its variants are absent in prosimians which are the most primitive primates, but evolved by deletion mutation in anthropoid primates, higher primates and human (Maston and Ruvolo, 2002). There has been evolution of hCG and its variants with increasing acidity and therefore longer circulation half life from lower primates to humans and this was thought to parallel the evolution of deeper implantation and placentation with improved nutrition of the foetus, needed to support the development of increasing size of brain to body ratio from early primates and other mammals, through anthropoid primates to advanced primates and humans (Martin, 1981, Rodesch *et al.*, 1992, Cunnane *et al.*, 1993, Martin, 1996, Pijnenborg, 1996, Gibbons, 1998, Burton *et al.*, 1999, Jauniaux *et al.*, 2000, Robillard *et al.*, 2003b, Jauniaux *et al.*, 2006). It is known that implantation of the blastocyst in humans is associated with high levels of CG-H, and is absent or is in low proportion in early pregnancy loss (Birken *et al.*, 2001). It has been demonstrated that a low proportion of CG-H accompanies failing human pregnancies from the time of implantation to miscarriage (Kovalevskaya *et al.*, 2002a, Sutton-Riley *et al.*, 2006). The fact that CG-H promotes trophoblast growth, invasion, and vascularisation, and the clear relationship between early pregnancy loss and low CG-H levels on one hand, and early pregnancy loss and poor implantation (Norwitz *et al.*, 2001), show the importance of CG-H in implantation (Cole, 2008). Based on these evidences, Cole et al (2008)have proposed that there is a correlation between evolution of hCG and CG-H with increasing numbers of oligosaccharides on hCG and CG-H, and evolution of deeper placental invasion, haemochorial placentation that is required to support brain development in evolution of primates and humans with increasing size of brain to body ratio (Cole, 2008). Pre-eclampsia is a significant pregnancy complication that is said to be maternal compensation for ineffective haemochorial placentation in early pregnancy (Pijnenborg, 1996, Robillard *et al.*, 2003a, Robillard *et al.*, 2003b, Burton and Jauniaux, 2004,

Jauniaux *et al.*, 2006, Goldman-Wohl and Yagel, 2008). The low CG-H circulating levels in the first trimester of women who developed pre-eclampsia in the third trimester has been reported (Bahado-Singh *et al.*, 2002). Exogenous CG-H administration in the first trimester has been proposed as a possible prevention of pre-eclampsia and early pregnancy loss (Cole, 2008). Measurement of urinary and blood CG-H levels in pregnancy could be the answer to the lack of an effective early diagnostic and screening test for pre-eclampsia.

IX) Expression of Major Histocompatibility Complex (MHC): Major histocompatibility genes are responsible for the recognition of non-self. Trophoblast cells are unique with regards to expression of MHC antigens. They do not express MHC class II antigens (Apps *et al.*, 2007). Moreover, trophoblast cells do not express Classical MHC class 1 antigens (HLA-A, -B and –D) involved in transplant rejection (Moffett-King, 2002, Trundley and Moffett, 2004). Some of the subtypes of extravillous trophoblast at the foeto-maternal interface express a unique set of MHC class I antigens, namely HLA-C, -E, -G and? F (Ellis *et al.*, 1990, Kovats *et al.*, 1990, King *et al.*, 1996, Loke *et al.*, 1997, McMaster *et al.*, 1998, King *et al.*, 2000b, Ishitani *et al.*, 2003, Nagamatsu *et al.*, 2006). Of these, only HLA-C, the classical Class I antigen, is polymorphic and is capable of generating alleles and may be important in allo-recognition of trophoblast (Moffett-King, 2002). HLA-G is expressed only by invasive EVTs (McMaster *et al.*, 1995). HLA-G is not polymorphic but different isoforms generated from alternative splicing exist (Hiby *et al.*, 1999, Ishitani *et al.*, 2003). There has been a report of some of these isoforms in amniotic fluid and they are said to be produced from an unusual glycosylation (McMaster *et al.*, 1998). The expression of HLA-G at the maternofoetal junctional zone is reduced in pre-eclampsia (Colbern *et al.*, 1994). HLA-E is a non-classical Class I antigen (King *et al.*, 2000a) and

it is expressed by HLA-G+ trophoblast (King *et al.*, 2000a, Ishitani *et al.*, 2003). The subtypes of EVTs in the basal plate differ with regard to the type of HLA antigens they express. Endovascular and interstitial trophoblasts express HLA -C, -E, and -G (Blaschitz *et al.*, 2001, Agrawal and Pandey, 2003). The mRNAs for these antigens is more widespread when compared to the distribution of the protein. Invasive trophoblast cells carry paternal genes and are therefore genetically different from maternal cells that they encounter at the maternofoetal zone. It has been a puzzle as to how they survive without being rejected based on the self/non-self recognition model proposed by (Medawar, 1953). It was thought that the HLA molecules they express are unable to provoke NK cell immune response due to lack of NK cell targets (King *et al.*, 1989, King *et al.*, 1990). The HLA-G is also thought to confer protection against cytolytic activity of the uterine NK cells (Chumbley *et al.*, 1994). Recent evidence however suggests that implantation may predominantly involve a yet-to-be-identified allogeneic recognition system involving uterine NK cells rather than T-cells (Loke and King, 1997, Hiby *et al.*, 2004).

Invasion of the Endometrium

The main function of the EVTs is invasion of the endometrium up to the superficial one-third of the myometrium. They cause remodelling of the uteroplacental vessels by replacing the endothelial cells and smooth muscle cells of the vessels walls (Kam, 1999). These vessels then become wide bore vessels that deliver a large amount of blood into the intervillous space at low pressure during the second half of pregnancy (Blankenship *et al.*, 1993, Kaufmann *et al.*, 2003, Goldman-Wohl and Yagel, 2008). They also interact with maternal NK cells and macrophages resulting in the production of cytokines and chemokines by maternal leucocytes. These compounds regulate trophoblast invasion of decidua and hence implantation.

Nitabuch's Stria

This layer of fibrinoid was described in 1887 by Nitabuch. It is located in the immediate area of the materno-foetal interface. This layer is rather more consistent than the Rohr's stria and forms a uniform layer of 20μm to 100μm thick (Benirschke and Kaufman 2000). Occasionally, it is interrupted by trophoblast, decidual cells and endometrial connective tissue. It is thought to represents the exact zone of interaction between maternal and foetal tissues, the so called "battlefield" (Wynn, 1967). There are more foetal tissues superficial to and more maternal tissues deeper to it in this ideal location. This arrangement is seen only in few places. A more common finding is a mixture of both cell types above and below the layer (Wynn, 1967). This layer of fibrinoid contains a mixture of both matrix-type and fibrin-type fibrinoid (Frank *et al.*, 1994, Lang *et al.*, 1994, Huppertz *et al.*, 1996, Huppertz *et al.*, 1998b). Both decidual (Azab *et al.*, 1972, Kisalus and Herr, 1988) and trophoblast cells secrete extracellular matrix into it (Frank *et al.*, 1994, Frank *et al.*, 1995, Huppertz *et al.*, 1996, Huppertz *et al.*, 1998b). The proposed function of this layer includes a protective role for the foetus by insulating it against maternal immune cells (Kirby *et al.*, 1964, Sutcliffe *et al.*, 1982). This view is supported by its relative absence in animals in which there is no invasive implantation on one hand and its rather dense presence in animals with invasive implantation (Sutcliffe *et al.*, 1982) and where there are big genetic differences between the mother and the baby (Kirby *et al.*, 1964). In pre-eclamptic placentae there is an increased deposition of immunoglobulins in the Nitabuch layer of the maternofoetal interface (Balducci *et al.*, 1997) indicating deposition of common antigens in the layer.

It may have a role in acting as an adhesive that attaches the placenta to the endometrium (Frank *et al.*, 1994). Other proposed functions include promotion of trophoblast invasiveness through the presence of the so-called cell spreading factor (Aplin and Foden, 1982).

The separation zone

This is the zone at which placental separation occurs. It is basal to the fibrinoid layer and it contains mainly decidual cells and components of endometrial stroma (Benirschke and Kaufman 2000). It also has elements of basal plate such as extravillous trophoblast cells and degenerating encased villi. There are more endometrial cells than foetal cells. As the distance from the intervillous space increases, the population of multinucleated giant cells increases (Hein, 1971, Pijnenborg *et al.*, 1980). After separation, the basal plate surface of the placenta is covered by fibrin. The decidua may play a role in placental separation as in clinical conditions such as extrauterine pregnancies or in placenta accreta where decidual cells are absent or reduced , placenta separation does not occur properly (Benirschke and Kaufman 2000).

Chorionic Plate

The chorionic plate forms the 'roof' of the intervillous space of the placenta and it is also the site from which villous trees are suspended into the intervillous space. It is the surface to which the umbilical cord is attached (Boyd and Hamilton, 1970, Benirschke and Kaufman 2000).

Development

The first recognised structure of the chorionic plate is the primary chorionic plate which is formed as early as day 8 post-conception. At this stage, it is a bilayer consisting of syncytiotrophoblasts and cytotrophoblast (Boyd and Hamilton, 1970, Jones and Jauniaux, 1995, Benirschke and Kaufman 2000, Moore *et al.*, 2008). The primary chorionic plate separates the early lacunae system from the blastocyst cavity. Later, the extraembryonic mesoderm is formed and spreads around the cytotrophoblastic surface of the blastocyst cavity. The primary chorionic plate is thus covered by the extra-embryonic mesenchyme which transforms it from a bi-layer into a tri-layer (Jones and Jauniaux, 1995, Benirschke and Kaufman 2000) (Fig. 1.4.). At this stage also, trophoblastic trabeculae which become the eventual stem villi grow from the primary chorionic plate into the expanding lacunae system. The structure of the triple-layered chorionic plate is maintained till term.

Tissue degeneration may however occur within the chorionic plate described above, thus disrupting the original architecture. Within the syncytiotrophoblast layer degeneration may occur at places with fibrinoid deposits replacing the degenerated tissues. The fibrinoid may be deposited along the surface and within the chorionic plate where it is called Langhan's fibrinoid (Bourne, 1962). This is matrix-type fibrinoid and it is compact and homogenous (Frank *et al.*, 1994, Benirschke and Kaufman 2000).

The cytotrophoblastic layer may also degenerate in places and replaced by fibrinoid. In others, it may also proliferate. The cytotrophoblast cells that maintain contact with the

chorionic mesoderm are proliferative while those distant from it become differentiated extravillous trophoblasts. These variants of extravillous trophoblast cells grow in size and they resemble invasive cytotrophoblast both in ultrastructure (Wiese, 1975) and histochemistry (Weser and Kaufman 1978).

Cavitation occurs in the extraembryonic mesoderm during the third week p.c. forming the exocoelomic cavity. This splits the extra embryonic mesenchyme into the chorionic and the amniotic mesenchymes, lining the primary chorionic plate and the amnion respectively (Boyd and Hamilton, 1970, Benirschke and Kaufman 2000, Moore *et al.*, 2008).

In the second month p.c, fetal blood vessels develop from allantois through the connecting stalk, and they gain access to the chorionic plate. These vessels run in the chorionic mesoderm before entering the stem villi. In these villi they establish contact with the locally developed fetal capillaries. This completes the fetoplacental circulation. The chorionic foetal vessels enlarge to form arteries and veins. Capillaries are rare in the chorionic plate (Boyd and Hamilton, 1970, Benirschke and Kaufman 2000, Moore *et al.*, 2008).

The amniotic vesicles enlarge greatly around the 17th day post-menstruation, bringing the amniotic epithelium and its mesoderm into contact with the primary chorionic plate. The amnion is made up of three layers at this stage; an inner amniotic epithelium, a mesoderm and an outer mesothelium. Where there is close contact between the amnion and the primary chorionic plate, their mesothelial layers fuse. There is usually a net-like bridge between these layers. This enables the two layers to glide over each other. The coming together and fusion of the primary chorionic plate and the amnion results in the formation of the definitive chorionic plate.

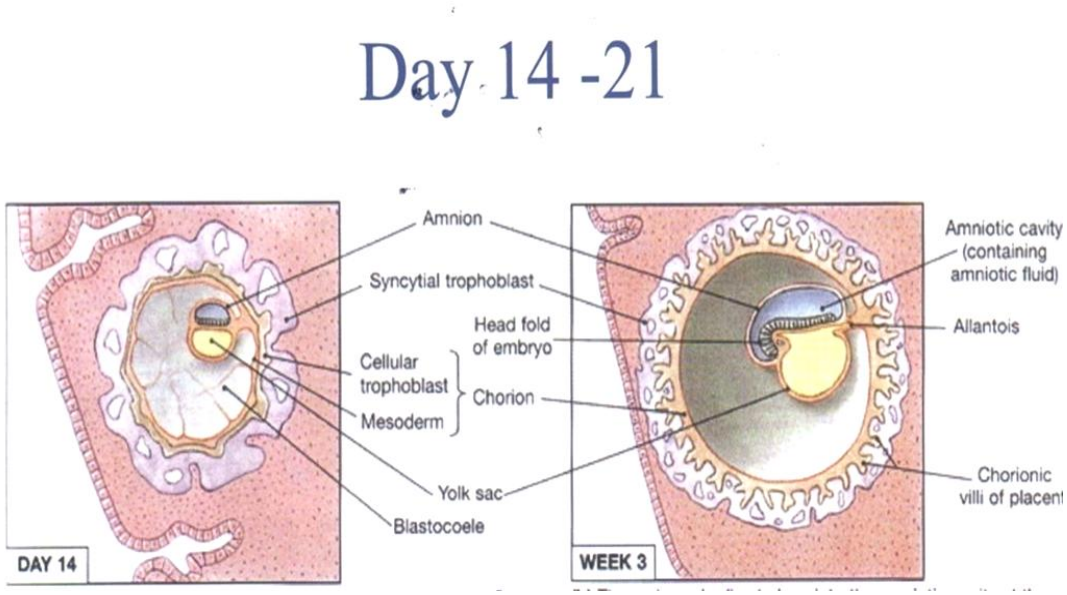


Fig. 1.4: Migration of mesoderm around inner surface of trophoblast results in a triple-layered chorion. This diagram is taken from Second EMBIC handbook, the European Network on Embryo Implantation Control (2008), Vol 1 (Szekeres-Bartho, J; Chaouat, G; and Joseph-Mathew, P), page 285

Term Chorionic plate

The layers of the Chorionic plate at term are similar to those of the amniochorion. The following layers of the chorionic plate have been described (Bourne, 1962, Bourne, 1966, Malak *et al.*, 1993, Ockleford *et al.*, 1993a, Fawthrop and Ockleford, 1994) and this is identical to its developmental layers. The layers are shown in the box 1.1and fig. 1.5 below. These layers are those present in the amnion and the chorion which make up the chorionic plate.

The Amnion

The amnion is made up of amniotic epithelium with its basement membrane, compact layer and amniotic mesoderm.

The amniotic epithelium is a simple cuboidal or columnar epithelium (Bourne, 1962, Bourne, 1966, Sinha, 1971, Malak *et al.*, 1993, Ockleford, 1993, Fawthrop and Ockleford, 1994). Squamous metaplasia is a common finding though, especially near the umbilical cord insertion (Sinha, 1971).

Box. 1.1: The layers of the chorionic plate at term

Amnion
- Amniotic epithelium
- Basement membrane of the amniotic epithelium
- Compact layer
- Amniotic mesoderm
- Spongy (intermediate) layer

Chorion
- Chorionic mesoderm
- Proliferating cytotrophoblast layer
- Langhan's fibrinoid with embedded invasive cytotrophoblast
- Syncytiotrophoblast

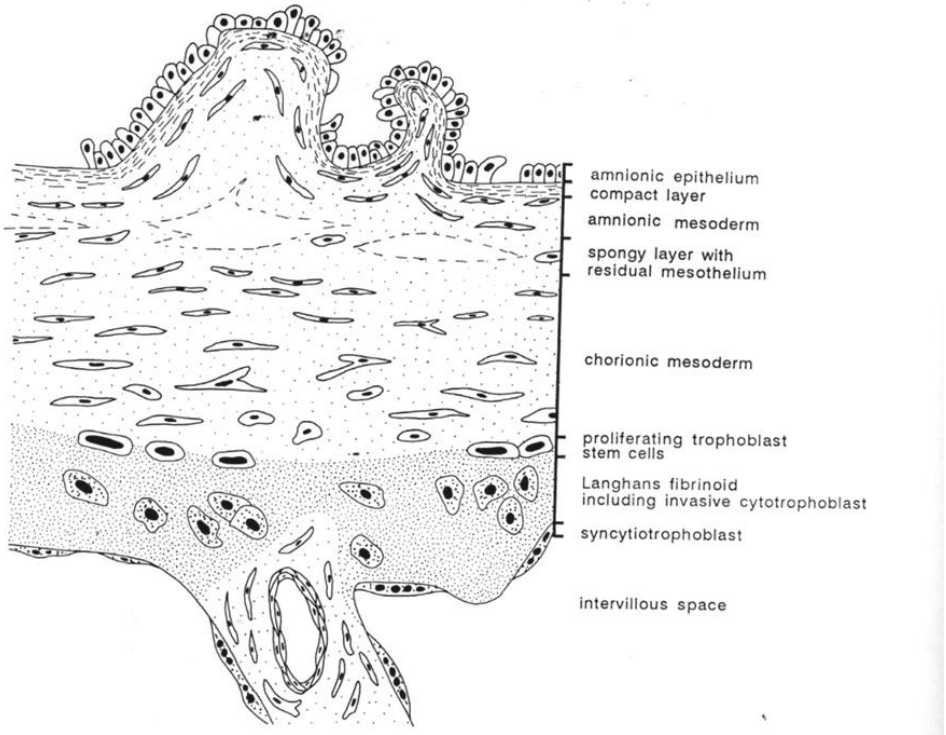


Fig. 1.5: A drawing showing the Layering and cellular composition of mature chorionic plate at term, an image from Pathology of the Placenta (2000), 4th ed. (Benirschke, K., and P. Kaufmann) Page 212.

The apical surface of the epithelial cells has projections of microvilli (Bourne, 1966, Lister, 1968, Fawthrop and Ockleford, 1994). The microvilli are infrequent and stunted in late amnion (Lister, 1968). Intracellular inclusions have been described at the ultrastructural level (Sinha, 1971, Fawthrop and Ockleford, 1994). The amniotic epithelial cells show primarily storage properties by having lipid droplets and glycogen in their cytoplasm (Sinha, 1971).

The cells have projections which anchor them onto the basement membrane. Also present are inter- and intracellular cannaliculae which are believed to play a role in transport cross the amniotic membrane (Sinha, 1971, Fawthrop and Ockleford, 1994). Desmosomes are the intercellular connections between the cells, both at the apical level and also act as interruptions along the intercellular channels (Sinha, 1971, Fawthrop and Ockleford, 1994). The base of the epithelial cells is thrown into folds that project into the basement layer (Lister, 1968). The amniotic epithelial cells express strong cytokeratin immunofluorescence (Malak *et al.*, 1993). They also express CD59 (Rooney and Morgan, 1992) and HLA- G (Rebmann *et al.*, 1999) which makes these cells resistant to transplant rejection even in allo-transplantation. Recently, human amniotic epithelial cells have been shown to express albumin, α1- antitrypsin and other hepatocyte-like genes and so they have been proposed to have potential role in liver transplant (Takashima *et al.*, 2004). The epithelial basal lamina is seen as a striated layer on the chorion side of the amniotic epithelial cells (Bourne, 1962, Bourne, 1966, Lister, 1968, Malak *et al.*, 1993, Fawthrop and Ockleford, 1994, Hu *et al.*, 2000). The striation is imparted by the infolding of the basal surface of the epithelial cells (Fawthrop and Ockleford, 1994). Like a typical basal lamina, it shows intense immunofluorescence with both monoclonal and polyclonal anti-collagen type IV and laminin antibodies (Malak *et al.*, 1993).

Compact layer

The compact layer is an acellular layer that is composed of closely packed collagen fibres arranged in a 3-dimensional lattice (Wiese, 1975). The corresponding layer of the amniochorion is said to have similar structure (Malak *et al.*, 1993, Ockleford, 1993, Fawthrop and Ockleford, 1994). Below the compact layer, amniotic mesoderm is also made up of loose collagen fibres that are arranged parallel to the amniotic epithelium (Lister, 1968, Fawthrop and Ockleford, 1994). Unlike the compact layer, it does not have vertically arranged fibres and the collagen fibres are intermingled with fibroblast cells (Malak *et al.*, 1993) hence, it also called the fibroblast layer. This fibroblast layer contains cells that co-express vimentin and desmin, indicating their myofibroblastic characteristics (Ockleford *et al.*, 1993b). This layer was therefore proposed to play a role in tension generation, adjustment in position of the membrane and wound healing (Ockleford *et al.*, 1993b). There are 3-4 layers of filaments that alternate with layers of fibroblast. It is known to contain an enzyme that is involved in production of extra-cellular matrix proteoglycans (Kratzsch and Grygiel, 1972).

The amnion has been used clinically in treatment of burns and repair of surgical wounds (Trelford and Trelford-Sauder, 1979), and in ocular reconstructive surgery (Koizumi *et al.*, 2000)

The Spongy layer

This is a layer of loose connective tissue that is formed by fusion of amnion and chorion when the amniotic and the chorionic mesoderm come together during development (Bourne, 1962). The spongy layer consists structurally of a fine fibrillar network (Malak *et al.*, 1993). It has spongy clefts that demarcate the zone of fusion between amnion and chorion. These clefts are less prominent in the chorionic plate as compared to the corresponding layer in the free foetal membrane (Benirschke and Kaufman 2000). In the amniochorion they are known to be lined by cells which are thought to be residues of the former amniotic and chorionic mesothelium (Malak *et al.*, 1993, Ockleford, 1993, Ockleford *et al.*, 1993b)

Chorionic mesoderm

This layer consists of connective tissue cells. Two distinct connective tissue layers have been described in this zone; the oriented connective tissue layer that is adjacent to the spongy layer and the unoriented connective layer that is near to the fibrinoid layer (Wiese, 1975, Weser and Kaufman 1978). The oriented layer is thinner and its collagen fibres are regularly arranged parallel to the spongy layer. In the unoriented layer, however, the collagen fibres are irregularly arranged with a large polymorphic fibroblast cells in between them. The connective tissue layers have been shown immunohistochemically to contain type I, III, IV, V and VI collagens (Malak *et al.*, 1993). It was suggested that the two layers represent different levels of differentiation and maturation during development with the unoriented layer being the primitive precursor (Wiese, 1975, Weser and Kaufman 1978). In support of the above assertion, it was found that there is predominance of the unoriented layer in first trimester placentae. The oriented layer on the other hand, contains the chorionic vessels. Bertolini et al. (1969)and Spanner (1935) reported the presence of smooth muscle cells within this layer, but this view was not supported by Wiese who studied this layer using electron microscopy (Benirschke and Kaufman 2000). Ockleford et al (1993b) have described cells which express desmin immunofluorescence in the reticular layer of the chorion laeve. Since desmin is characteristic of muscle, they suggested that these cells of the mesodermal origin are myofibroblastic . They have therefore suggested mechanical and wound healing functions of this layer. This myofibroblastic differentiation was supported by the finding that there is increase in the numbers and density of vimentin-positive, α-smooth muscle actin-expressing mesenchymal cells of the reticular layer of the chorion in the lower uterine pole biopsy in labour-affected and rupture line biopsies (McParland, 2000). Cellular activities associated with this transformation may play a role in membrane rupture and onset of labour (McParland, 2000).

Fibroblast cells of the chorionic plate were found to express caveolin-1 isoforms, a protein that plays a role in the transport across these cells (Byrne *et al.*, 2001).

Trophoblast cells

The chorionic plate contains a layer of trophoblast cells located deep to the chorionic mesoderm (Bourne, 1960, Wiese, 1975, Wiese, 1976, Weser and Kaufman 1978). A trophoblast basal lamina also known as pseudo basement membrane is located deep to the chorionic mesoderm (Bourne, 1960, Malak *et al.*, 1993, Ockleford, 1993, Fawthrop and Ockleford, 1994). In places devoid of extravillous cytotrophoblast layer, the basal lamina is absent (Benirschke and Kaufman 2000).

The layers of trophoblast cells are not consistent throughout the entire chorionic plate. In the centre of the placenta, these cells are in small and focal groups that are part of a discontinuous layer but towards the placental margin, they form a continuous multilayer of cells (Wiese, 1975, Weser and Kaufman 1978). The cells in the layer are described as being in different stages of differentiation of the same cell (Wiese, 1975). The trophoblast cells in the multilayer interdigitate with each other, with desmosomes being the type of cell-to-cell junctions. Foot-like processes anchor the cells to the basal lamina through formation of hemidesmosomes. Where cytotrophoblast cells are absent they are replace by fibrinoid and degenerated cells (Wiese, 1975).

Fibrinolytic components such as tissue-type plasminogen activator (tPA), urokinase-type plasminogen activator (uPA) and their corresponding inhibitors, type-1 (PAI-1) and type-2 (PAI-2) and their messenger RNAs were identified in the trophoblast cells of the chorionic plate and extravillous cytotrophoblast cells and at other locations in human and rhesus monkey placenta (Hu *et al.*, 1999).

The intervillous surface of the chorionic plate is formed by syncytiotrophoblast, cytotrophoblast and intercellular material especially fibrin (Wiese, 1975, Wiese, 1976, Weser

and Kaufman 1978). The intervillous surface of the chorionic plate that roofs the marginal sinus of the placenta was reported to be partially lined by endothelial cells (Nanaev *et al.*, 2000). Stem villi project from the chorionic plate into the intervillous space and occupy part of the intervillous surface of the chorionic plate.

Langhan's Fibrinoid Layer

The fibrinoid layer of the chorionic plate is present in both normal and pathological placentae (Hormann, 1965). Compared to preterm placentae, there is increased deposition of fibrinoid in late gestation placentae (Frank *et al.*, 1994). In the preterm placenta, the intervillous surface of Langhan's layer is covered entirely by syncytiotrophoblasts, but as pregnancy advances towards term, part of the syncytium is replaced with additional fibrinoid (Benirschke and Kaufman 2000).

The characteristics of Langhan's fibrinoid are similar to the fibrinoid of the basal plate described above. The amount of chorionic plate fibrinoid at term varies from placenta to placenta. There is no defined amount that is compatible with normal pregnancy (Benirschke and Kaufman 2000). A very prominent layer was noticed in Rh incompatibility. In such cases Langhan's fibrinoid stria may occupy about half the volume of the placenta (Benirschke and Kaufman 2000). Deposition of this fibrinoid results in formation of bosselations and laminated subchorial plaques. At times, large subchorionic thrombohaematomas may be found underneath the fibrinoid (Shanklin and Scott, 1975, Olah *et al.*, 1987). These are similar to the classical Breus mole which were described in early pregnancy placentas (tuberous subchorial haematoma) except that Breus mole has sacculations.

Pre-eclampsia

Pre-eclampsia (PE) is a very common and potentially dangerous complication of the second half of pregnancy, labour and puerperium. The disease is one of the major causes of maternal and foetal mortality and morbidity in both developed and developing countries. It is defined as development of **hypertension** and **proteinuria**, usually after 20 weeks of gestation (or can occur earlier in trophoblastic diseases like hydatiform mole) in a previously normotensive pregnant woman (ACOG, 2002).

Epidemiology

Pre-eclampsia occurs worldwide but the exact incidence of pre-eclampsia is not known. The reported incidence varies greatly, probably due to differences in case definition, population composition, demographic and obstetric characteristics and true variation in incidence (WHO, 1988). It affects 5-8% of all pregnancies (Saftlas *et al.*, 1990, Hauth *et al.*, 2000) and 2.5-3.0% of all women suffer from pre-eclampsia in their pregnancy (Redman and Sargent, 2005). In the United States, hypertensive diseases occur in approximately 12-22% of pregnancies (Walker, 2000). Pre-eclampsia is one of leading causes of maternal and perinatal morbidity and mortality. In developed countries such as the United States of America and United Kingdom maternal mortality has declined, but pre-eclampsia is still one of the important causes of maternal death. In the United Kingdom for example, it is one of the leading causes of maternal mortality, responsible for about 10 maternal deaths every year (de Swiet, 2000). In developing countries where prenatal care is still poor for various reasons, pre-eclampsia and other hypertensive disorders of pregnancy accounts for 10% to 15% of all maternal deaths (Duley, 1992). In the Greater Accra Region of Ghana, a typical developing country, hypertensive disorders of pregnancy, including pre-eclampsia, accounts for 22.2% of maternal deaths (Zakariah *et al.*, 2006). It also causes about 1000 perinatal deaths per year in the United Kingdom (Acolet *et al.*, 2005). Much of the neonatal mortality and morbidity

following pre-eclamptic pregnancy is due to iatrogenic immaturity since the only cure for the disease at the moment is delivery of the baby (refer to management of pre-eclampsia).

Risk factors

Pre-eclampsia occurs commonly in nulliparous women. A number of foetal and maternal risk factors have been identified for pre-eclampsia. In a systematic review, Duckitt and Harrington (2005)have identified the following risk factors of pre-eclampsia that may be present at antenatal booking (Box 2.1): These are multiple pregnancies, nulliparity, previous history of pre-eclampsia, presence of antiphospholipid antibodies, family history, chronic hypertension, raised blood pressure at booking, pre-existing maternal diabetes mellitus, renal disease, raised maternal body mass index before pregnancy or at booking, maternal age 40years or more for multiparous women, interval of 10years or more between previous and current pregnancy, and autoimmune diseases (Duckitt and Harrington, 2005). They proposed the use of these factors for determining pregnant women at risk of developing pre-eclampsia at antenatal booking visits.

Box. 1.2: Risk factors for pre-eclampsia

Maternal

- Nulliparity
- Pre-eclampsia in previous pregnancy
- Advanced maternal age (>40years)
- Family history of Pre-eclampsia
- Chronic hypertension
- Chronic renal disease
- Antiphospholipid antibody syndrome or inherited thrombophilia
- Autoimmune vascular or connective tissue disease
- Diabetes mellitus
- Abnormal body mass index

Fetal

- Multi-foetal gestation
- Hydrops foetalis

Pre-eclampsia is classified as mild or severe based on the clinical severity.

Criteria for severe Pre-eclampsia

It is considered severe when any one or more of the features listed in Box 1.3 is present in addition to a new onset of proteinuric hypertension (ACOG, 2002). The disease is classified as mild when none of these criteria is present.

Box. 1.3. Criteria for severe pre-eclampsia. American College of Obstetricians and Gynecologist (ACOG) Criteria for classification of PE as Severe-ACOG technical bulletin number33/International Journal of Gynecology & Obstetrics 53 (19%) 175-183

1) Blood pressure > 160-180 mm Hg systolic or >110 mm Hg diastolic.

2) Proteinuria > 5 g/24 h (normal = 300 mg/24 h)

3) Elevated serum creatinine

4) Grand mal seizures (eclampsia)

5) Pulmonary oedema

6) Oliguria < 500 ml/24 h

7) Microangiopathic hemolysis

8) Thrombocytopenia

9) Hepatocellular dysfunction (elevated alanine, aminotransferase, aspartase aminotransferase)

10) Intrauterine growth retardation or Oligohydramnios

Two broad types of pre-eclampsia: maternal and placental has been described (Redman and Sargent, 2005) based on the cause. In the placental type the problem arises from an

abnormally formed placenta that is hypoxic and oxidatively stressed (Burton and Jauniaux, 2004). In the maternal type, a well formed placenta interacts with abnormal maternal constitutional conditions such as chronic systemic hypertension and diabetes mellitus to produce pre-eclampsia (Ness and Roberts, 1996).

Pathophysiology of Pre-eclampsia

The exact cause of pre-eclampsia is not known, but placenta is said to be the source of the disease as the presence of placenta with or without a foetus is required for the disease to develop and it resolves completely after delivery of the placenta (Redman, 1991). Pre-eclampsia is described as maternal reaction to an abnormally formed placenta or pre-existing maternal systemic conditions.

Placental pre-eclampsia

The placental type has been described as a two-staged disease: Preclinical and clinical (Redman and Sargent, 2005). The preclinical stage starts early in pregnancy at the time of formation of the placenta with no clinical symptoms (Fig. 1.6). During normal pregnancy, uterine spiral arteries which are end-arteries are remodelled by the invasive extravillous cytotrophoblast (EVT) cells. These cells invade the uterine lining from the 6^{th} to the 18^{th} week of gestation (Red-Horse *et al.*, 2004). These uterine arteries are plugged by endovascular cytotrophoblast cells until about the 9th week of gestation (Hustin *et al.*, 1988, Burton *et al.*, 1999) when they begin to recanalize, a process which is completed by the 12^{th} week of gestation (Hamilton and Boyd, 1960, Harris and Ramsey, 1966, Jauniaux *et al.*, 2000). This is associated with an increased placental oxygenation (Burton *et al.*, 1999) and extensive remodelling of the placental spiral arteries by endovascular and interstitial EVTs which replace the endothelial cells and cause destruction of the muscular and elastic elements of the media with fibrinoid deposition. This is termed physiological change (Brosens *et al.*, 1967). The remodelling involves the distal myometrial segment of the arteries as well

(Pijnenborg *et al.*, 1983). When the process is completed at about 20 weeks of gestation, the spiral arteries are converted into wide-bore vessels with loss of vasomotor tone that deliver large amount of blood at low pressure into the intervillous space (Brosens *et al.*, 1967). In the placental type pre-eclampsia, there is failure, or incomplete remodelling, of the arteries (Brosens *et al.*, 1972, Matijevic and Johnston, 1999, Burton and Hung, 2003). This is called poor placentation and it occurs before 20 weeks of gestation and before the clinical signs of the disease begins to appear. Poor placentation alone, however, may not be responsible for causing the disease. The characteristic features of poor placentation are seen in some pregnancies with growth restricted foetuses without clinical pre-eclampsia (Khong *et al.*, 1986).). Other materno-foetal factors are probably involved.

The cause of poor placentation has been the subject for many studies over the years (Zhou *et al.*, 1997). Zhou et al (1997) have reported that there is failure of endovascular cytotrophobblast cells to mimic vascular adhesion cell phenotype in pre-eclampsia.

Immune mechanism is said to be involved in the pathophysiology of the disease. Immune rejection of the paternally derived fetal genes by the mother has long been suggested as a cause of pre-eclampsia (Medawar, 1953). Epidemiological evidences of immunologic involvement are: increased risk in primigravidae, multiparae with partner change (Trupin *et al.*, 1996) and increased risk in women who received egg donation (Söderström-Anttila *et al.*, 1998). Hiby et al (2004) have suggested a weaker trophoblast histocompatibility antigen to decidual immune cell signalling as responsible for the poor placentation .

Uterine NK cells which are the predominant lymphoid cells (King *et al.*, 1998) express receptors that bind to HLA-C antigens on the trophoblast cells (Vilches and Parham, 2002). These receptors are called killer cell immunoglobin-like receptors (KIRs) (Moretta *et al.*, 1995, Selvakumar *et al.*, 1997, Martin *et al.*, 2000). Based on gene content, there are two haplotypes of the KIRs, A and B with A being simpler. A mediates inhibitory signals whilst

B is more complex and involved in stimulatory signals (Hsu *et al.*, 2002, Shilling *et al.*, 2002). All HLA-C alleles can also be grouped into two KIR epitopes: C1 and C2 (Colonna *et al.*, 1993, Mandelboim *et al.*, 1996). HLA C2 binds with higher affinity to KIRs than HLA-C1 (Parham, 2005). The fact that NK cells have receptor that can bind to the trophoblast HLA-C means that there may be interaction between the two cells and the HLA-C-KIR ligand-receptor complex may mediate immune recognition and control of trophoblast invasion of the uterine decidua and spiral arteries (Hiby *et al.*, 2004).

It has been shown that women who are homozygous for the A type haplotype (AA) are at an increased risk of developing pre-eclampsia and the risk is higher when the fetus they carry is also homozygous for the C2 type HLA-C (Hiby *et al.*, 2004). In other words, there is less trophoblast stimulation of the decidual NK cell in women who are at an increased risk of developing PE.

During the second half of pregnancy (stage two) when there is increased demand for blood supplies into the intervillous space of the placenta to support the developing fetus the poorly formed placenta, with inadequate utero-placental circulation, becomes hypoxic (Kaufmann *et al.*, 2003) and oxidatively stressed through intermittent flow causing perfusion and reperfusion injury (Hung and Burton, 2006). This abnormal placenta then releases pro-inflammatory and antiangiogenic compounds into the maternal circulation to cause the maternal syndrome (Redman and Sargent, 2009).

The clinical features of the disease are ascribed to maternal reaction to generalised endothelial dysfunction (Roberts, 1998) which is part of the generalised systemic inflammatory response in pregnancy that is exaggerated in pre-eclampsia (Redman *et al.*, 1999). This then causes a triad of circulatory disturbances namely; generalised maternal vasospasm, local or generalised enhanced intravascular coagulation and reduced plasma volume (Redman and Sargent, 2005). Generalised maternal endothelial dysfunction has been

proposed as the immediate cause of these physiologic derangements (Roberts *et al.*, 1989, Redman *et al.*, 1999). This proposal has since been confirmed by many workers who have identified clinical and biochemical evidences of endothelial dysfunction (Taylor *et al.*, 1991, Hsu *et al.*, 1993, Friedman *et al.*, 1995). Endothelial dysfunction results in excessive production of or increased sensitivity of blood vessels to vasoconstrictors such as endothelins, serotonin and angiotensin converting enzyme II, and or decreased production of or reactivity to vasodilator agents like nitric oxide and prostacylin. This eventually results in generalised increase vasomotor tone, increase peripheral resistance, hence the increased maternal blood pressure (BP) seen in the disease. Endothelial dysfunction causes glomerular endotheliosis, which is one of the consistent feature of the disease (Farquhar, 1959, Spargo *et al.*, 1959, Gaber *et al.*, 1994), making them more permeable and so there is net filtration of protein into the urine, hence the proteinuria.

Intravascular coagulation is a feature in pre-eclampsia and is associated with platelets activation (Janes and Goodall, 1994, Konijnenberg *et al.*, 1997) thrombocytopenia and often, reduced antithrombin III (Horn *et al.*, 1991). There is also plasma volume contraction in the disease and this is due to vasospasm, increased leakiness of capillaries with resultant leakage of plasma fluid into the interstitium (Brown *et al.*, 1989) and in severe cases, reduction in plasma oncotic pressure (Oian *et al.*, 1986). In effect, there is a net flow of fluid from the intravascular compartment into the interstitial space but without a change in the extracellular fluid volume (Brown *et al.*, 1992). Increased vasospasm resulting in increased peripheral resistance, low plasma volume, and enhanced blood clotting with formation of microthrombi will lead to hypoperfusion of almost all organs. The organs most commonly affected are kidneys (manifesting as reduced glomerular filtration rate, proteinuria, hyperuricaemia and in severe cases, oliguria), the liver (raised liver enzymes, oedema of the hepatocytes and stretching of the liver capsule), brain (scotoma from frontal lobe ischaemia, severe headache,

seizure in some cases and cerebrovascular accidents) and placenta (intrauterine growth restriction, small for gestational age babies, abruption placenta and intra uterine foetal death).

The best known of the factors released by the abnormal placenta is soluble vascular endothelial growth factor receptor-1 (sVEGFR-1) also known as soluble fms-like tyrosine kinase-1 (sFlt-1). Several workers have reported evidence that excess secretion of this placental-derived anti-angiogenic factor is the cause of the maternal syndrome (Koga *et al.*, 2003, Maynard *et al.*, 2003, Sugimoto *et al.*, 2003, Tsatsaris *et al.*, 2003, Chaiworapongsa *et al.*, 2004, Levine *et al.*, 2004). It is produced in trophoblast cells and secreted into maternal circulation (Banks *et al.*, 1998, Clark *et al.*, 1998) and the excess production is stimulated by hypoxia and oxidatively stressed trophoblast cells in the placenta (Maynard *et al.*, 2003, Nevo *et al.*, 2006). Soluble VEGFR-1 is a variant of VEGFR-1, but it lacks the transmembrane and cytoplasmic domain. It binds to angiogenic factors, vascular endothelial factor (VEGF) and placental growth factor (PIGF) and inhibits their action (Kendall and Thomas, 1993). This decreases the concentration of these molecules in the circulation; hence the endothelium is deprived of essential growth factors making them dysfunctional.

Clear evidences exist that seems to incriminate sFlt-1 in the pathogenesis of the maternal syndrome. Among these is the finding that there are increased levels of the gene products of sFlt-1 in pre-eclamptic placentae (Zhou *et al.*, 2002, Maynard *et al.*, 2003, Tsatsaris *et al.*, 2003). The increase in the concentration of sFlt-1 in the maternal circulation in pre-eclampsia is accompanied by decrease in the level VEGF and PlGF, a state which is reversible after delivery, coinciding with resolution of clinical symptoms of the disease (Zhou *et al.*, 2002). Maynard at al. (2003) have also shown that the sera of pre-eclamptic patients have anti-angiogenic effects that are reversible in the presence of excess VEGF and PlGF. Furthermore, it has been demonstrated that transfection of rats with sFlt-1 induces pre-eclampsia-like features which includes hypertension, proteinuria and glomerular

endotheliosis which is regarded as the pathognomonic lesion of pre-eclampsia (Maynard *et al.*, 2003). It has also been shown that glomerular-selective deletion of the VEGF-A from the podocytes resulted in proteinuria and glomerular endotheliosis in mice (Eremina *et al.*, 2003). Lastly, it has been shown recently that the state of increased sFlt-1 concentration and decreased concentration of VEGF and PlGF predates the appearance of clinical symptoms in pre-eclampsia (Levine *et al.*, 2004). sFlt-1appears to have a role in the pathogenesis of the maternal syndrome and not only that, its anti-angiogenic effect is also said to probably play a part in the deficient development of the utero-placental vessels in the disease (Karumanchi and Bdolah, 2004).

Soluble endoglin is another antigiogenic factor that is said to be release by the syncytiotrophoblast and its circulating levels are increased in pre-eclampsia (Venkatesha *et al.*, 2006). Hypoxia stimulates its production (Yinon *et al.*, 2008).

Other proinflammatory compounds that are released from the placenta include corticotrophin releasing hormone (CRH) (Perkins *et al.*, 1995), Activin-A (Muttukrishna *et al.*, 1997), Inhibin-A (Muttukrishna *et al.*, 1997), Leptin (Mise *et al.*, 1998). Placental growth factor and trophoblast microparticles (Redman and Sargent, 2008). They are known to affect maternal systemic angiogenesis and or systemic inflammation (Redman and Sargent, 2009)

Another factor that has been credited with causing the syndrome in the mother is Neurokinin B. It has been found to produce similar features of the disease in animal studies (Page *et al.*, 2000).

During pregnancy the placenta releases trophoblast tissues into the maternal circulation (Chua *et al.*, 1991). Recent reports have suggested that this trophoblast debris plays a role in the systemic inflammatory response associated with pregnancy (Borzychowski *et al.*, 2006). This debris comprises syncytiotrophoblast membrane particles, cytokeratin fragments,

soluble DNA and RNA of foetal origin and cytotrophoblast cells. The release of these particles from the syncytial surface occurs in all pregnancies but is increased in pre-eclampsia more than in normal pregnancy (Knight *et al.*, 1998). Excess shedding of these syncytiotrophoblast microparticles is said to be the feature of early onset pre-eclampsia but not pure intrauterine growth restricted foetal pregnancies (Goswami *et al.*, 2006), an evidence of its role in the pathogenesis of the disease. It has been suggested that the trophoblast deportation associated with pre-eclampsia is more likely to be of necrotic origin (Burton and Jones, 2009). Hypoxia and oxidative stress that characterises pre-eclampsia are a stimulant for apoptosis and necrosis (Huppertz *et al.*, 2003). Pre-eclampsia is associated with systemic oxidative stress. This together with the placental effect of oxidative stress has inspired clinical trials for the use of anti-oxidant vitamin C and E in the prophylactic treatment of the disease. Recent results show they are not beneficial in preventing pre-eclampsia in high risk women (Poston *et al.*, 2006, Villar *et al.*, 2009).

A new finding has revealed that there is down-regulation of cytokeratin immunofluorescence in chorionic villi of pre-eclamptic placentae compared to normal pregnancy placenta (Ockleford *et al.*, 2004). The removal of such debris from the maternal circulation constitutes an immune burden and hence the systemic inflammation that characterises the disease (Redman and Sargeant, 2003). That trophoblast debris deported into the maternal circulation constitutes an inflammatory stimulus explains why, epidemiologically the disease risk is higher and is often more severe in the presence of excess trophoblast tissue as in multiple pregnancy and in hydatiform mole (with deregulated proliferation of trophoblast cells). It may also explain why pre-eclampsia is predominantly a disease of the third trimester.

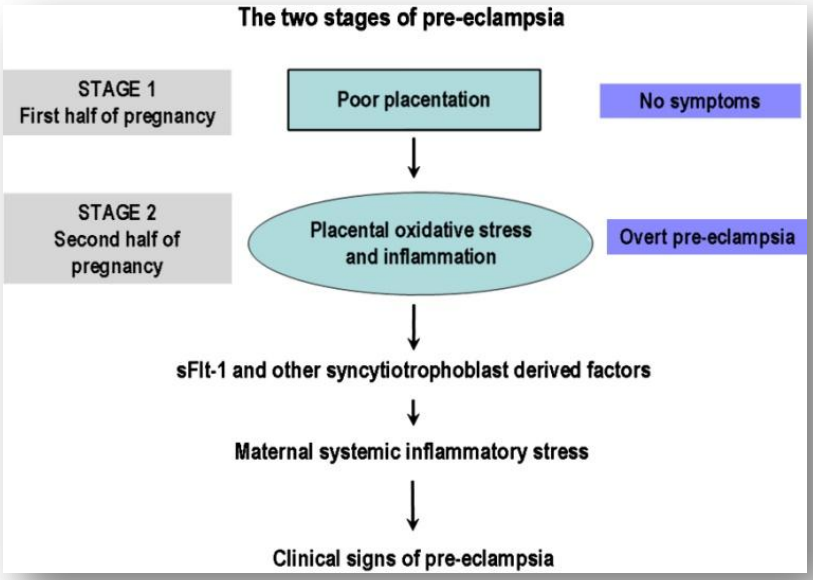


Fig. 1.6: This is a chart showing the two-staged model of pre-eclampsia proposed by Redman CWG and Sargeant IL (2009). Placental oxidative stress and maternal systemic inflammatory responses are the two central features.

Maternal pre-eclampsia

In this type of pre-eclampsia a normal placenta interacts with an abnormal systemic constitution resulting in the disease (Ness and Roberts, 1996). These abnormal maternal conditions include obesity, hypertension, vascular diseases, and diabetes. In the presence of pregnancy those affected go on to develop pre-eclampsia.

Clinical Manifestations

Pre-eclampsia is largely asymptomatic for most part of its clinical course (Redman, 1992, ACOG, 2002). When symptoms and signs do appear they do so at a late stage of the disease. The signs and symptoms are also not specific to the disease. These characteristics of the disease make it difficult to diagnose, and early treatment is almost impossible. The clinical symptoms and signs are listed in the box 1.4 below:

Hypertension

It is generally the earliest sign that raises the suspicion of the presence of the disease. Currently, it and proteinuria are the features used for screening for the disease in antenatal attendants. Hypertension in pregnancy is defined as a systolic blood pressure (SBP) ≥140 mm Hg or diastolic blood pressure (DBP) of ≥90 mm Hg in pregnant woman after 20 weeks of gestation (Roccella, 2000) Usually in pregnancy, blood pressure decreases in the first trimester and then rises again in the second trimester until term as part of the normal physiology of pregnancy (Morgan, 1991). The rise associated with the second half does not usually reach a hypertensive level. In pre-eclampsia there is disturbed endothelial control of vascular tone leading to increased vasomotor tone, increased peripheral resistance, and therefore hypertension (Roberts, 1999).

Proteinuria

Proteinuria is defined as the presence of 0.3 g or more of protein in a 24-hour urine specimen or 1+ or greater in a random urine dipstick evaluation (Roccella, 2000). Proteinuria is usually a late finding in the disease. Urinary excretion of protein increases gradually in pregnancy and in pre-eclampsia it often reaches a range 5 g or more. It is due to impaired glomerular barrier and renal tubular handling of filtered proteins leading to increase protein in the urine (Moran *et al.*, 2004).

Weight gain

Weight gain is a feature of pregnancy. This physiological weight gain is usually gradual. Weight gain of 0.4536 Kg per week is normal while a weight gain of 0.9072 Kg in one week or a total weight gain of 6 pounds in a month is abnormal and it is a sign of pre-eclampsia (Redman, 1992). Sudden increase in weight rather than gradual increase is characteristic (Redman, 1992).

Box. 1.4. Clinical features of pre-eclampsia.

Hypertension
Proteinuria
Hyperuricaemia
Excessive weight gain
Excessive oedema
Thrombocytopaenia
Increased serum lactate dehydrogenase
Right upper abdominal or epigastric pain
Elevated liver transaminases
Subcapsular haematoma
HELLP syndrome
Headache
Blurred vision
Scotoma
Stroke
Pulmonary oedema
Foetal growth retardation
Oligohydramnios
Abruptio placentae

Oedema

Oedema is common in pregnancy. It is therefore no longer regarded as a diagnostic criterion for pre-eclampsia (ACOG, 2002). However, excessive oedema involving the face and the fingers can be a clue of the presence of pre-eclampsia (Redman, 1992). Oedema is due to redistribution of fluid in the extracellular compartment.

Central Nervous system

Severe pre-eclampsia and eclampsia may affect the central nervous system (CNS). CNS manifestations include headache, blurred vision, scotoma (Roccella, 2000) and hyperreflexia (ACOG, 2002). Headache is not specific to pre-eclampsia and therefore other causes should be ruled out before ascribing it to preeclampsia. Scotoma is impaired or loss of visual acuity in some part of the visual field. Occasionally, severe pre-eclampsia may be associated with temporary blindness. This is however uncommon (Cunningham *et al.*, 1995). Intracranial

haemorrhage has been reported as the cause of death in some severe preeclampsia-eclampsia (Richards *et al.*, 1988).

Right upper abdominal or epigastric pain.

Preeclampsia patients may complain of right upper abdominal or epigastric pain. This is pain from stretching of the hepatic capsule as a result of hepatocellular oedema and occasionally, there is also subcapsular haematoma as well (Sheehan and Lynch, 1973). Liver involvement may be indicated by HELLP syndrome which is raised liver transaminases in the circulation, low platelet count and haemolysis (Sibai, 1990, Sibai, 2004).

Pulmonary oedema

Various factors may be responsible for pulmonary oedema in pre-eclampsia. Elevation of pulmonary vascular hydrostatic pressure above oncotic pressure will lead to net movement of fluid from the vascular compartment into the alveoli resulting in pulmonary oedema. This phenomenon is not present in all cases of pre-eclampsia with pulmonary oedema (Benedetti *et al.*, 1985). Other causes of pulmonary oedema in preeclampsia are fluid overload from intravenous fluid administration, left heart failure and pulmonary capillary dysfunction.

Fetal Symptoms

Intra-uterine Fetal Growth Restriction

The fetus sometimes bears the effect of the placental hypoperfusion in pre-eclampsia in the form of intra-uterine fetal growth retardation (IUGR). The risk of small for gestational age infant following preeclampsia is four times higher than normal pregnancy (Odegard *et al.*, 2000). In severe pre-eclampsia the mean birth weight is reduced by 12% whilst early onset disease decreases the birth weight by 23% compared with normotensive pregnancy (Odegard *et al.*, 2000).

Oligohydramnios

Reduced amniotic fluid volume is another manifestation of pre-eclampsia. This is the result of reduced utero-placental blood supply in the disease.

Abruptio placentae

Early separation of the placenta from the uterus before birth of the baby is termed abruptio placentae. It presents as antepartum haemorrhage and may be complicated by postpartum haemorrhage as a result of clotting derangement. It is infrequent (1%) in mild pre-eclampsia but it has been reported in 3% of severe pre-eclampsia cases (Sibai *et al.*, 1994)

Diagnosis

The diagnosis of pre-eclampsia can be very difficult because all its symptoms occur late and they are not specific to the disease. Moreover, the early pathology of the disease is not accessible and not fully understood (Redman, 1992). Hypertension and proteinuria are the characteristic clinical features that are used in diagnosis of the disease at the clinic (Sibai, 2003). The diagnosis is based on finding hypertension and proteinuria in a pregnant woman after 20 weeks gestation if they were previously normotensive (Roccella, 2000).

Criteria for diagnosis of pre-eclampsia are as follows:

a. Hypertension: systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg.

b. Proteinuria: 0.3 g (300 mg) or more protein in a 24 hour urine specimen (ACOG, 2002).

c. The elevation in blood pressure (BP) should be sustained. Hence for this to be regarded acceptable, two measurements must be taken, at least six hours apart but not more than seven days (Roccella, 2000).

Blood pressure measurement

The BP measurement should be taken after sitting quietly for 5 minutes or more, after physical activity (O'Brien *et al.*, 2005). The reading should be taken with appropriate cuff size. The length of the cuff bladder should be 80% and the width, 40 % of the length of the circumference of the upper arm (Pickering *et al.*, 2005). Others recommend 46 % (Beevers *et*

al., 2001). The arm should be at the level of the heart (O'Brien *et al.*, 2005) and ideally the patient should be in sitting position (Jamieson *et al.*, 1990). The diastolic BP should be recorded at the Korotkoff phase V (disappearance of sound) (Beevers *et al.*, 2001, ACOG, 2002). The old diagnostic criteria of 30 mm Hg or more increase in systolic BP and or 15 mm Hg or more increase in diastolic BP above the early pregnancy or the booking values even if the BP is below the hypertensive levels of 140/90 mmHg is no longer regarded as suitable (ACOG, 2002). This is because this increment has low sensitivity and predictive value, about 30% for both. They are usually not associated with adverse outcomes (North *et al.*, 1999, Levine *et al.*, 2000).

Screening for proteinuria

Screening for protein in the urine is done using a urinary dipstick. Equivalent protein concentrations in the urine and the corresponding urinary dipstick measurement values are show in the table 1.1 below. 1+ positive reaction on dipstick corresponds to a threshold concentration of 30 mg /100 ml of urine which is equivalent to 300 mg in 24-hour urine when the urine volume is 1000 ml. Dipstick measurement is not very accurate in determining the severity of proteinuria since protein concentration is a function of urine volume and the amount of protein in the urine (Meyer *et al.*, 1994). Dipstick is qualitative and is prone to give false negatives and false positives due to differences in osmolality (Gribble *et al.*, 1995). Other factors that can cause false positive dipstick readings are blood in the urine, very alkaline urine, quaternary ammonium compounds, detergents, disinfectants certain drugs and high specific gravity urine (> 1.030). Similarly, low specific gravity urine (< 1.010), high salt concentration, highly acidic urine or non albumin protein in the urine can cause false negative readings (Meyer *et al.*, 1994, Gribble *et al.*, 1995).

Waugh et al (2004) have reported that 1+ reaction is of no clinical value since negative dipstick does not necessarily exclude significant proteinuria as positive result does not

necessarily mean significant proteinuria. Quantitative methods should be used to confirm a positive dipstick reaction. A 24-hour urine collection with measurement of protein content is usual and appropriate. It has the advantage of providing information for calculation of glomerular filtration rate (GFR) if creatinine concentration is also measured. Urine protein to creatinine ratio is a simple and fairly accurate option. It relies on random urine specimen for determination of urine protein-to-creatinine ratio which accounts for differences in osmolality (Ginsberg *et al.*, 1983). It has been reported to have a high correlation coefficient (r =0.93) in both ambulatory and hospitalized patients when compared to 24- hour urine collection results (Neithardt *et al.*, 2002). There is however, no agreed threshold for identifying women with significant proteinuria (Young *et al.*, 1996, Rodriguez-Thompson and Lieberman, 2001, Neithardt *et al.*, 2002, Durnwald and Mercer, 2003). A value of 0.14 to 0.19 is said to be significant (Young *et al.*, 1996, Rodriguez-Thompson and Lieberman, 2001, Neithardt *et al.*, 2002, Durnwald and Mercer, 2003).

Table 1.1: Urinary dipstick results and the corresponding urinary concentrations

Dipstick protein	Protein concentration (mg / dl)
Trace	15-30
1+	30-100
2+	100-300
3+	300-1000
4+	≥1000

Differential Diagnosis

The diagnosis of pre-eclampsia must be confirmed by excluding other conditions in which hypertension and proteinuria are present. Clinical and laboratory findings must be used to distinguish PE from other conditions that present similarly. Conditions that present like pre-

eclampsia therefore can pose diagnostic dilemmas. These include: gestational hypertension, pre-existing hypertension, and primary and secondary renal diseases like nephritic syndrome and glomerulonephritis. Other conditions causing coagulopathy, hypertension, abnormal liver function and renal abnormalities in pregnancy must also be excluded too. These include acute fatty liver of pregnancy, thrombotic thrombocytopenic purpura, haemolytic uraemic syndrome, exacerbation of systemic lupus erythematosis (SLE), gestational thrombocytopenia, autoimmune thrombocytopenia, cerebral haemorrhage, migraine, hepatitis, cholestasis and pancreatitis (Sibai, 2007) (Box 1.5). PE is the commonest cause of hypertension, proteinuria, coagulation abnormalities and derangement in liver enzymes in pregnancy but the above conditions must be born in mind and excluded as well (Sibai, 2007).

Box. 1.5 Differential diagnosis of Pre-eclampsia

Gestational hypertension
Pre-existing hypertension
Primary and secondary renal diseases
Acute fatty liver of pregnancy,
Thrombotic thrombocytopenic purpura
Haemolytic uraemic syndrome
Exacerbation of SLE
Gestational thrombocytopenia
Autoimmune thrombocytopenia
Cerebral haemorrhage
Migraine
Hepatitis
Cholestasis
Pancreatitis.

Laboratory investigations.

Laboratory evaluation of women with PE involves tests to aid diagnosis, to determine severity, and to monitor progress so as to detect maternal and foetal complications. Below are some of the laboratory tests that have been recommended by the National High Blood

Pressure Education Program Working group on high blood pressure in pregnancy (Roccella, 2000).

Urine Protein measurement

Urinary protein of 300 mg in 24hour urine is diagnostic (Roccella, 2000, ACOG, 2002). When 5 g or more protein is excreted in the urine, pre-eclampsia is severe. 1+ dipstick protein must be confirmed using 24 hour urine collection or protein to creatinine ratio (ACOG, 2002).

Haematocrit

It measures the red cell mass. The haematocrit may be low, high or normal in PE. If it is low it may indicate haemolysis. On the other hand, haematocrit may be high due to haemoconcentration which may be a feature of severe disease.

Platelets counts

Thrombocytopenia is common in pre-eclampsia. Platelet count of $\leq 100000/ mm^3$ connotes severe pre-eclampsia or may be part of HELLP syndrome (Sibai, 2004). Thrombocytopenia is due to widespread intravascular blood clotting that characterises the disease, with the resultant depletion of platelets.

Lactate dehydrogenase (LDH) Concentration

LDH is an enzyme that is involved in red blood cell (RBC) metabolism. When there is red cell lysis as in haemolysis, LDH is released into the circulation and the concentration is elevated (Marchand *et al.*, 1980). Elevated LDH indicates haemolysis which may be part of HELLP syndrome. High LDH concentration in the presence of high haematocrit suggests haemoconcentration.

Peripheral Blood Smear

Peripheral blood smears may show fragmented RBCs. These appear as spherocytes and helmet cells in peripheral blood film. It suggests microangiopathic haemolysis (Sibai, 2004).

Blood Urea and Creatinine

Serum urea and creatinine level may be elevated in the disease. This is due to reduced renal blood flow leading to reduced GFR. An elevated serum creatinine (>80 μmol/l) concentration or rising levels will suggest worsening severity.

Serum Uric Acid

Serum uric acid level is high and the level rises with advancing pre-eclampsia. Uric acid determination helps to differentiate PE from chronic hypertension.

Liver Function Tests (LFTs)

An elevated or rising level of alanine (ALT) and aspartate aminotransaminase (AST) signifies severe PE. It indicates liver involvement in the disease. If γ-glutaryltransaminase and bilirubin are also raised then HELLP syndrome is present.

Coagulation Tests

Prothrombin time and activated partial thromboplastin times are usually normal in pre-eclampsia unless there in an associated low platelet count or liver dysfunction. It is therefore not recommended to monitor this test routinely (Barron *et al.*, 1999).

Non- stress Test

This is the use of a cardiotocograph to monitor foetal heart beat pattern. Signs of abnormality include absence of acceleration, deceleration and lack of variability.

Biophysical Profile

In this test ultrasonography is used in looking at foetal breathing movements, limb movements, defining good posture and amniotic fluid volume. Each parameter is scored at a maximum 2 and minimum 0. A total score of 2 to 4 is ominous whilst 6 to 8 indicates good foetal wellbeing.

Management

The safety of the mother and the delivery of a mature baby is the main objective for the management of pre-eclampsia (Sibai, 2003). Delivery of the baby and the placenta, which removes the pathogenic focus, remains the only cure for pre-eclampsia currently (Roccella, 2000, ACOG, 2002). Even though delivery is favourable to the mother, it may not be so for the baby (Norwitz and Repke, 2007). Therefore, management should involve early diagnosis and appropriately timed delivery (Redman, 1992). Diagnosis should be made by screening for hypertension and proteinuria at the antenatal clinic and looking out for risk factors (see risk factors) (Redman, 1992, ACOG, 2002). The initial assessment should aim at determining the severity of the disease, maternal and foetal condition, and presence of associated complications. This assessment involves history, physical examination, laboratory investigations and foetal non-stress testing and determination of biophysical profiles. Once the diagnosis is confirmed and the initial assessment is completed, there are two management options:

1. Active (aggressive) with delivery of the baby
2. Expectant.

Which option is decided on, is based on the gestational age of the fetus, the severity of the disease, maternal and fetal condition, presence of labour, cervical Bishop Score and availability of materno-fetal supportive services (Lain and Roberts, 2002, Sibai, 2003).

Aggressive management

This is the delivery of the baby after stabilization of the mother. Patients with mild pre-eclampsia at term (37 completed weeks), must be delivered (Roccella, 2000, Norwitz and Repke, 2007). Most experts recommend that the baby must be delivered by 40 weeks when pre-eclampsia is mild (Roccella, 2000, Sibai, 2003, Nicholson *et al.*, 2006). Severe pre-eclampsia is also generally regarded as an indication for delivery (Norwitz and Repke, 2007). These include those with end-organs involvement and also those with other obstetric complications such as preterm rupture of membrane and abruptio placentae or the presence of labour, and non-reassuring non-stress test results (see box 1.6, indications for delivery). One important indication for the delivery of the baby at any gestational age is maternal hepatic subcapsular haematoma (Lain and Roberts, 2002). This is because of the risk of hepatic rupture (Lain and Roberts, 2002).

Expectant management

This option is delaying delivery for some time. The aim is to achieve some degree of foetal maturity so as to reduce complications associated with preterm delivery e.g. foetal distress syndrome, asphyxia, hypothermia and neonatal infections, etc. Expectant management involves close monitoring of both mother and baby so as to identify any complications that develops and then early and appropriate interventions are applied when needed.

Expectant management is indicated for patients with mild disease remote from term and some selected cases of severe pre-eclampsia (Lain and Roberts, 2002, Sibai, 2003, Norwitz and Repke, 2007, Sibai and Barton, 2007). These patients are monitored closely for development of complications and appropriate and timely intervention taken. It is now known that complications are rare in mild pre-eclampsia patients provided they are compliant (Sibai, 2003, Norwitz and Repke, 2007). Out-patient management is said to have similar pregnancy outcomes to that of in-patient management and is therefore recommended. Hence, after the initial diagnosis and evaluation, patient could be followed on an outpatient basis which is

more cost effective (Barton *et al.*, 1994, Barton *et al.*, 2006). Many experts have also recommended conservative management for selected severe cases (Odendaal *et al.*, 1990, Sibai *et al.*, 1994). In a recent review, Sibai and Barton(2007) have outlined the selection of the appropriate candidates for expectant management of severe pre-eclampsia. The selected patient must not have signs of any end-organ involvement i.e. eclampsia, pulmonary oedema, acute renal failure, disseminated intravascular coagulation, HELLP syndrome, thrombocytopenia, labour, premature rupture of membrane (PROM), severe foetal growth restiction and non-reassuring foetal status. The gestational age for these patients must be between 24 and 32 weeks.

Bed rest is thought to reduce oedema, encourage foetal growth and prevent worsening hypertension and therefore better pregnancy outcomes (Gilstrap *et al.*, 1978, Sibai *et al.*, 1987, Sibai *et al.*, 1992a). However there is no evidence that complete bed rest improves pregnancy outcomes (Goldenberg *et al.*, 1994). Restricted activity is typically recommended.

Fetal and maternal monitoring during conservative management

Laboratory investigations

As part of maternal monitoring, laboratory tests are done frequently to detect increasing severity, and development of complications (end-organ involvement) to both mother and baby. These tests include platelet counts, serum ALT and AST, serum creatinine, GFR, and urinary protein excretion (Roccella, 2000). There are no obvious values for other laboratory investigations such as haematocrit, serum LDH and urinary protein quantification. It is known that neither the rate of increase nor the amount of protein affects maternal or perinatal outcome (Schiff *et al.*, 1996, Hall *et al.*, 2002). Fetal assessment is important in determining fetal well-being and to identify complications. This is done by fetal movement counts, non-stress test (NST) and ultrasonographic assessment of fetal activity and amniotic fluid volume, also known as biophysical profile (BPP). The American College of Obstetricians and

Gynaecologists have recommended a minimum of daily fetal movement counts and weekly or bi-weekly NST and BPP (ACOG, 2002). When fetal movement count is found to be abnormal, NTS and BPP could be than more frequently (Roccella, 2000). Ultrasonography to measure fetal weight is important in detecting fetal growth restriction which may be a sign of PE or progression to severe disease. Ultrasound should be done at diagnosis and should be repeated serially.

Antihypertensive therapy

The effect of antihypertensive drug therapy on the length of pregnancy, on foetal growth, and on the incidence of preterm delivery varies. There are no clear benefits of antihypertensive treatment in mild pre-eclampsia (Sibai, 1996, Barton *et al.*, 1999). Antihypertensive treatment is important when diastolic blood pressure is between 105-110 mm Hg or more (Sibai, 1996, Barton *et al.*, 1999, Roccella, 2000). It reduces the incidence of certain complications, like cerebrovascular accidents from occurring. When treatment is indicated, the drug that is chosen must reduce the blood pressure to a safe level, have a rapid action, reduce the pressure in a controlled manner without lowering cardiac output and utero-placental blood flow and it must be safe for both mother and the baby (Roccella, 2000, Alexander *et al.*, 2006). Currently the drugs that are used in hypertensive crisis include Hydralazine, Labatalol, Nifedipine and Sodium Nitroprusside (Roccella, 2000). Methyldopa is also thought to be safe and is also used in the treatment of hypertension in pregnancy.

Box. 1.6: .Dosage of Antihypertensive drugs in pre-eclampsia

1. Hydralazine: 5-10 mg intravenously every 15-20 minutes until the desired BP is reached effective; 80 mg at 10 minute intervals until a maximum total dose of 220 mg is reached.
2. Labatalol: 20 mg intravenous bolus followed by 40 mg within 10minutes if no adequate response.

Anticonvulsant Therapy

Anticonvulsant drugs are administered to prevent seizure in women with severe pre-eclampsia (Coetzee *et al.*, 1998, ACOG, 2002, Roberts *et al.*, 2002). Its role as seizure prophylaxis in mild pre-eclampsia is uncertain (Witlin *et al.*, 1997, Hall *et al.*, 2000, Magpie Trial Collaboration, 2002, Livingston *et al.*, 2003, Alexander *et al.*, 2006). Anticonvulsant therapy is usually given during labour or just before a planned delivery and is continued 12 to 48 hour after delivery (Norwitz and Repke, 2007).

Currently, parenteral Magnesium Sulphate ($MgS0_4$) is the drug of choice for seizure prevention in pre-eclampsia (Lucas *et al.*, 1995) and prevention of recurrent seizure in eclampsia (Duley and Henderson-Smart, 2003b). It is more effective than other anticonvulsant drugs such as phenytoin, diazepam and lytic cocktail (which is a mixture of chlorpromazine, promethazine and pethidine) that have been used in the past (Duley and Gulmezoglu, 2001, Duley *et al.*, 2003, Duley and Henderson-Smart, 2003b, Duley and Henderson-Smart, 2003a).

Corticosteroid Therapy

Infants born prematurely due to pre-eclampsia are prone to hyaline membrane disease (Chang *et al.*, 2004) which is due to immaturity of the lungs. It has been shown that administration of corticosteroids to the mother less than 32 weeks gestation, 24 to 48 hours before delivery, prevents this by promoting foetal lung maturation (Amorim *et al.*, 1999).

Delivery

It is the only cure for the disease. The indications for delivery in pre-eclampsia are summarised in the box 1.7 blow:

Box. 1.7: Indication for delivery adapted from report of the national high blood pressure education program working group on high blood pressure in pregnancy volume 183 (1), 2000.

Maternal	Gestational age≥38 weeks
	Platelet count <100 000cells/mm^3
	Progressive deterioration in renal function
	Progressive deterioration in hepatic function
	Suspected abruptio placentae
	Persistent severe headache or visual changes
	Persistent severe epigastric pain, nausea, or vomiting
Fetal	Severe foetal growth restriction
	Non-reassuring fetal testing results
	Oligohydramnios

Route of Delivery

The route of delivery depends on each individual patient (Lain and Roberts, 2002). Mild pre-eclampsia at term should be delivered vaginally provided there are no contraindications (Norwitz and Repke, 2007). Vaginal delivery avoids an additional stress of operative delivery and is therefore preferred to caesarean delivery (Roccella, 2000). Labour can be induced when the cervix is favourable (Bishop Score of 6 or more). Cervical ripening agents can be considered when the cervix is not favourable. Vaginal delivery should also be attempted in most severe cases of pre-eclampsia too, particularly those beyond 30 weeks gestation (Sibai, 2003). On the other hand, when pre-eclampsia is severe and the gestational age is below 30 weeks or there are foetal and maternal complications such as fetal distress, foetal growth retardation then speed of delivery is important and caesarean delivery is preferred (Sibai, 2003). Caesarean delivery is also performed for other obstetric indications (Roccella, 2000). The risk of caesarean delivery must always be weighed against the advantages gained by speed of delivery.

Complications of Pre-eclampsia

Pre-eclampsia is a disease with unpredictable course and it can progress at great speed with development of complications within a short time of the onset, hence making it dangerous and very difficult to manage (Redman, 1992). Complications of the disease are due to end organ involvement particularly kidney, liver, brain and the lung. Some of the major adverse outcomes include eclampsia (pre-eclampsia with seizure), stroke owing to cerebral haemorrhage, HELLP syndrome, subcapsular haematoma with possible hepatic rupture, disseminated intravascular coagulation (DIC) and thrombocytopenia with bleeding tendencies, renal failure, pulmonary oedema, intrauterine foetal growth retardation, abruptio placentae, and perinatal death (Roccella, 2000). Factors that influence these outcomes include gestational age at onset and delivery, severity of the diseases, and presence of co-morbid conditions like multiple gestation, diabetes mellitus, renal failure, thrombophilia or pre-existing chronic hypertension (Heard *et al.*, 2004).

Pre-eclampsia–eclampsia is also an important cause of maternal and perinatal morbidity and mortality. The case fatality rate of pre-eclampsia –eclampsia is about 6.4 per 100 000 live births (MacKay *et al.*, 2001, Livingston *et al.*, 2003).

Mild pre-eclampsia is said to have comparable neonatal outcome to that of normotensive pregnancies except for higher induction rate (Hauth *et al.*, 2000). Severe pre-eclampsia on the other hand is associated with significant neonatal and maternal complications (Hauth *et al.*, 2000).

Long term outcomes

Hypertension and proteinuria resolves after delivery. This is usually within days but it may sometimes take weeks. Some patients with severe hypertension will need antihypertensive treatment postpartum. If hypertension persists after 12 weeks then it is probable that pre–

eclampsia was a misdiagnosis and may warrant referral to physicians for management (Roccella, 2000).

Patients who develop PE during pregnancy carry a risk of developing cardiovascular conditions like hypertension, ischaemic heart disease and stroke in the future (Sibai *et al.*, 1986, Irgens *et al.*, 2001, Smith *et al.*, 2001, van Walraven *et al.*, 2003, Funai *et al.*, 2005, Bellamy *et al.*, 2007). The risk is higher in those with early onset disease, severe disease and recurrent pre-eclampsia and gestational hypertension in multipara (Sibai *et al.*, 1986, Irgens *et al.*, 2001). This group probably have latent predisposing conditions like hypertension, thrombophilia or genetic or environmental factors.

The disease has the tendency to recur during subsequent pregnancies. Various factors determine the risk of recurrence. Among these is gestational age at onset and the severity of the disease. Women with severe pre-eclampsia are at great risk (25 to 65 %) of developing the disease in subsequent pregnancy when compared to women who had mild disease (5 to 7 %), and normotensives (1%) (Campbell *et al.*, 1985, Sibai *et al.*, 1986, Sibai *et al.*, 1991, Sibai *et al.*, 1992b, Xiong *et al.*, 2002). The risk of recurrence is about 65% when severe disease occurs in the second trimester (Sibai *et al.*, 1991) and the subsequent pregnancies are characterised by severe pre-eclampsia compared to nulliparous women.

Aims and objectives

The intervillous space of human placenta receives oxygenated maternal blood from spiral arteries that open onto the basal plate surface. The blood in the intervillous space is in direct contact with the chorionic villi. Exchange of nutrients, gases and waste products between the fetus and the mother occurs by simple diffusion across the materno-fetal barrier consisting of syncytiotrophoblast and its basement membrane, cytotrophoblast, and foetal capillary endothelium. The intervillous space which is enclosed between the basal plate and the chorionic plate is drained by maternal veins. The intervillous space therefore, is an extension of the maternal vascular space. The basal plate and the chorionic plate intervillous surfaces lining cells are similarly thought of as an extension of maternal endothelium. The basal plate intervillous space is lined by trophoblast cells of foetal origin and endothelial cells of maternal origin. Part of the lining is also covered by fibrin type fibrinoid. The chorionic plate intervillous space lining was described as composed of trophoblast cells. Endothelial cell dysfunction and generalised maternal systemic inflammatory reaction are well known features of pre-eclampsia, a very common and potentially dangerous complication of the second half of pregnancy. The disorder is also associated with clotting derangement. The aims of this study are:

(1) To find out the effect of severity of pre-eclampsia on the proportions of the basal plate intervillous surface lining cells.

(2) To find out whether the endothelium forms part of the lining of chorionic plate intervillous surface.

2 MATERIALS AND METHODS

Patient recruitment

Approval for the study was obtained from the Leicestershire Research Ethical Committee. Ref 7144 and the University Hospitals of Leicester NHS Trust Research and Development Committee Ref UHL9161. In collaboration with the consultants at the Pregnancy Hypertension Unit of the Leicester Royal Infirmary (LRI), suitable candidates for the study were identified during admission to the ward. The criteria for selection included: clinical diagnosis of pre-eclampsia supported by laboratory investigations, caucasian race and nulliparity. Multiparity, multiple pregnancies and women with maternal medical conditions such as hypertension and diabetes were excluded from the study. Candidates, who qualified, were recruited after a fully informed consent procedure was administered and agreement in writing obtained. These candidates were then monitored until they delivered, either normally or by caesarean section. Placental basal plate samples were collected within an hour of delivery.

A total of 32 placenta samples, comprising of eleven (11) normal pregnancy placentae, ten (10) mild pre-eclamptic (MPE) pregnancy placentae and eleven (11) severe pre-eclamptic (SPE) placentae were collected for the basal plate study. The classification of pre-eclampsia into mild and severe is based on the American College of Obstetricians and Gynaecologists (ACOG) criteria (refer to box1.3). Eight (8) healthy control patients were recruited for the chorionic plate study.

Sampling Method

Basal plate sampling

Four basal plate samples were randomly obtained from each placenta. Each sample is about 1 cm x 2 cm, and they were cut from each of the four quadrants of the placenta (as shown in

Figure 3.1) within an hour of delivery of the placenta. The rest of the placenta was disposed of by the hospital staff according to the prescribed protocol.

Chorionic plate sampling

For three (3) of the seven placentas recruited for the chorionic plate study, one random chorionic plate specimen, 1cm x 2cm in size, was sampled. For the other four (4) placentas, an 1 cm × 2 cm samples each were cut from the edge, midway between the edge and the centre of the placenta and near the umbilical cord insertion from each placenta.

Tissue processing

The samples were placed on wet ice in a Dewar flask and transported to the laboratory. In the laboratory, the samples were then immersed in OCT embedding medium (Tissue-Tek, Sakura Finetek Europe) in aluminium foil moulds and then freeze-fixed in a Dewar flask containing a slush of liquid hexane and dry ice. The frozen specimens, were placed in labelled mini freezer bags, and then transferred into a -80^{o}C freezer for storage until they were processed.

Placental samples for histology were fixed in 10% formaldehyde within an hour of delivery for 24-48 hours. The tissues were then trimmed into about 2 cm by 3cm in size and then dehydrated in graded series of ethyl alcohol and then cleared in Xylene before they were embedded in paraffin wax using a tissue processor (kindly provided by MRC Toxicology Unit, University of Leicester). Paraffin embedded tissues were kept in dry place until processed.

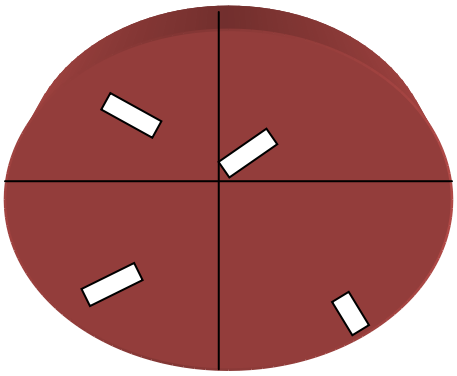


Fig. 2.1: A diagram showing the tissue sampling from the basal plate surface of the placenta. A basal plate sample was cut from each of the four quadrants of the placenta.

Dual-labelled Indirect Immunohistochemistry

Tissue sectioning

7µm-thick sections of the samples were cut using a Bright cryomicrotome. The temperature of the microtome cabinet was set at -25^{o}C and the tissue temperature at -13^{o}C. The tissue blocks were mounted on the chuck in the cryostat using OCT. The ends of the tissues were trimmed, with the cutting thickness of the microtome knife initially set at 20µm, until tissue of the basal plate was visible. The thickness was readjusted to 7µm and the sections cut and picked on subbed super frost slides (VWR International bvba, Haasrode Researshpark Zone 3, Geldenaaksebaan). The tissue slides were then fixed in 1:1 Acetone-methanol at room temperature in a Coplin jar for between 5 and 10 minutes. After fixation, the slides were washed 5 times, each wash lasting 5 minutes in 20mM Tris Buffered Saline with 0.1% Tween 20 (TBS-T)in a Coplin jar.

Primary antibodies

Trophoblast markers: Mouse monoclonal anti-pancytokeratin-IgG1 specific (Sigma-Aldrich, Missouri, USA, C2931) and rabbit (sc-17031-R) polyclonal anti-keratin 18 (p-Cytokeratin 18 Ser 33) (Santa Cruz Biotechnology, Inc.). The endothelial markers: Rabbit polyclonal anti-human von Willebrand factor (Sigma-Aldrich, Missouri, USA, F 3520) and mouse

monoclonal antibody raised against umbilical cord endothelial cells of human origin, Endothelium (EN4) (Santa Cruz Biotechnology Inc.). The antibodies were diluted in TBS-T containing 20% foetal calf serum, 1:800 for the mouse monoclonal anti-pancytokeratin, 1:100 for the EN4, 1:400 for the polyclonal rabbit anti-human von Willebrand factor and 1:50 for the rabbit polyclonal anticytokeratin 18. These dilutions were ascertained by titration.

Secondary antibodies

The secondary antibody for the trophoblast marker was Cy3-conjugated sheep anti-mouse Affinipure IgG Fab2 (Jackson Immunoresearch Laboratories, West Grove, PA, USA, 51787) at a dilution of 1:1000 and that for the endothelium marker is FITC conjugated goat-anti-Rabbit IgG(Sigma-Aldrich, Missouri, USA, F6005) at a dilution of 1:500. These optimal dilutions were arrived at after preliminary experiments were carried out to determine the optimum working dilutions.

Application of the antibodies to the slides

After washing slides in TBS, they were dried with tissue paper to remove excess water. The outlines of the tissues on the slides were marked out using a slide marker pen. This formed a water resistant barrier around the tissue and retained the antibodies dropped onto the tissue. The tissue on the slides were then flooded with 100µl of the primary antibody preparation and then incubated at 4^{o}C overnight in a humid container so as to prevent desiccation of the tissues.

Following an overnight incubation, the slides were give a further five washes, each 5 minutes, in 20mM TBS-T). Subsequently, 100µl of secondary antibodies were dropped on the slides to cover the tissue and the slides were incubated for 1-2 hours at room temperature in a dark cabinet.

At the end of the second incubation period, the slides were given a further five washes in 20mM TBS-T, each lasting five minutes, and then the slides were covered with glass

coverslips using Mowiol an acqueous mountant (from Calbiochem) containing the anti-photobleaching agent Diazabicylo [2.2.2] octane (DABCO).

Control experiment

Two types of control experiments were carried out to rule out false positivity as a result of non-specific binding of the primary antibodies. In the first type of control experiment, the primary antibodies were omitted and replaced by 20% foetal calf serum. The rest of the experiment was as described for the experimental group. In the second type of control experiments, isotype control antibody was applied instead of the primary antibodies and at the same dilution. Again, the rest of the experiment was as described above for the main experiment.

Epifluorescence Microscopy

The slides were examined under a Zeiss Epifluorescence microscope that was fitted with standard filter sets for Cy-3 and FITC fluorophores, to determine whether the slides are properly labelled and to see the general outline of the tissue. Slides with specific labelling and good tissue morphology were selected for confocal laser scanning microscopy.

Confocal Laser Scanning Microscopy (CLSM)

The tissue sections were viewed using the CLSM in a dual setup where 2 filters (green and red) were used. The slides were viewed under epifluorescence illumination to locate the basal plate. The Zeiss Axiovert was reset to allow the laser illumination through the confocal light path to the tissue. With the laser source set at zero, gain set at 7 for the red channel and 8 for the green channel, objective lens at x 20 magnification and using a Kalman filter at setting 3, the tissue was initially scanned with minimum pixel dwell time to minimise photobleaching. Simultaneous dual channel illumination on both channels and slow scanning speed filtering was used to record digital micrographs. In this way the tissue was scanned from one end of the basal plate to the other and images were recorded in a continuum along

the basal plate and saved on an optical disk under a specified file format (.pic) for further processing. This was done for each placenta. Fig3.2 is an example of a pair of typical micrographs obtained and saved. The identical process was applied to the chorionic plate samples.

Measurement

Using Comos software, the images obtained were merged side-by-side using pseudocolor-red and green coding for ease of identification of the different antigens. Green (FITC-fluorophore) was associated with cells expressing von Willebrand protein, and red (Cy3 fluorophore) labelled cells expressing the cytokeratin proteins. The basal plate lining was, at some places, interrupted by anchoring villi and gaps, not labelled by either antibody. These are designated as anchoring villi (AV) and non-specifically labelled (NS) respectively

Using the length/profile tool of the Comos software, the length of the red and green overlays, AV and NS along the basal plate lining of the intervillous space for each placenta was measured and recorded as the length of trophoblast (TPB), endothelium (END), AV and NS in microm. These data were transferred to an Excel spread sheet and then saved for analysis. The percentage length of the various components was then calculated. In three placentas no anchoring villous was seen in the fields examined. The lowest AV length measured for the group of each of those cases were used in place of zero.

Histology

Tissue sections, 5 µm thick, were cut using a microtome and sections placed in a drop of distilled water on a warm glass slide. After about 2-5 minutes the excess water was blotted away and the tissue slide left to dry out on hot plate for 3 hours. The tissue slides were then dewaxed by passing through Xylene first and then through a graded series of methanol (100%, 90% and 70%) and then water. A modified version of the staining method described

by Lendrum et al.(1962) was used. Cell nuclei were stained with Celestine blue and the slides rinsed under tap water. Nuclei were differentiated in 0.25 per cent hydrochloric acid in 70 % alcohol. The tissues slides were then washed in tap water and then rinsed in 95 % alcohol before they were stained with 0.5 % martius yellow in 95 % alcohol containing 2 % phosphotungstic acid for 20 seconds. The slides were then rinsed in 95 % ethanol and then stained in 0.1 % brilliant crystal scarlet 6R in 2.5 % acetic acid for 3 minutes. The slides were then rinsed in distilled water and treated with 1 % phosphotungstic acid for 5 minutes in order to fix and differentiate the red stain. The slides were then rinsed with distilled water and then stained with 0.5 % soluble blue in 1 % acetic acid for up to 3 minutes. Finally the slides were rinsed in 1 % acetic acid, blotted and dehydrated in absolute alcohol before being cleared in xylene. The tissues were coverslipped with synthetic resin as mounting medium.

Principle of the MSB staining

Fibrin is an insoluble fibrillar protein formed by polymerization of fibrinogen. The small molecular dye, martius yellow, together with phosphotungstic acid in alcohol solution stains red cells. The early fibrin maybe coloured but the phosphotangstic acid blocks the staining of muscle, collagen and connective tissue fibres. Brilliant crystal scarlet, a medium sized molecule, stains muscle and mature fibrin. Phosphotangstic acid removes any red stain from the collagen. The large molecule dye, celestine blue stains the collagen and old fibrin. Fibrin stains red (early fibrin may colour yellow and very old fibrin blue), red blood cells stain yellow, collagen stains blue, nuclei stain blue and muscle pale red (Cook, 1974, Bancroft and Stevens, 1982).

Statistical analysis

The graphs displaying the data were generated in Excel (Microsoft office 2007) (Fig. 5.1-5.3) and SPSS (SPSS version 16). Kolmogorov-Smirnov and Shapiro-Wilk tests (SPSS version 16), were used to determine normality of the data. ANOVA and Kruskal-Wallis (SPSS version 16) were used to test the differences between the means and median respectively for the length percent of endothelium, trophoblast, fibrin/fibrinoid and anchoring villi segments of the basal plate intervillous surface lining within and between the patient groups.

3 RESULTS

Demographic and obstetric characteristics of the study group

Maternal age, gestational age at delivery, sex of the babies and birth weight distributions of the study groups are summarised in Table 3.1 below. The average age of mothers in the severe pre-eclampsia group is 22.9 years. The average ages of women in the mild pre-eclamptic and healthy control groups are 27 years and 24.7 years respectively. Maternal age is not significantly different amongst the study groups (f (2, 29) = 1.967, p = 0.158)

The mean birth weight for the babies in the study group varies significantly between the patients groups (F (2, 29) = 11.912, p =0.000) (Table 3.2 and Table 5.10). The mean birthweight of babies born to mothers with severe pre-eclampsia is signicantly lower that those born to women who developed mild pre-eclampsia or had healthy pregnancies, posthoc p = 0.000 and 0.001 for mild pre-eclamptic and healthy controls respectively. Seven of the 11 babies born to mothers who had pregnancies complicated by severe pre-eclampsia were females and 4 were males (Table 3.1 and fig. 5.6)). In the mild pre-eclamptic group, 6 of the 10 babies were females and the other 4 were male babies (Table 3.1 and fig. 5.5). Mothers with healthy uncomplicated pregnancies had 5 female and 6 male babies (Table 3.1 and fig. 5.4). There is no significant differences among the groups with regards to the gender of the babies (p = 0.686). The mean gestational age at delivery was significantly different amongs the groups (Anova p = 0.001). Pregnancies complicated by severe pre-eclampsia were, as expected, terminated at an earlier gestational age (mean gestational age at delivery of 34.8 weeks) than normal pregnancies (p=0.001) and mild pre-eclamptic pregnancies (p = 0.003) (Table 3.1).

Table 3.1 Demographic and obstetric characteristics of the patient groups

	Control patients n=11	Mild pre-eclampsia n=10	*Severe pre-eclampsia* *n=11*
Maternal Age	24.7 (19-35)	27.0 (17-31)	*22.9(17-30)*
Gestational age at delivery (weeks)	38.7 (31-41)	38.5 (37-40)	*34.8 (31-39)*
Birthweight(g)	3057.1 (1980-4138)	3200.7 (2636-4110)	*2004.3 (1020-3628)*
Sex of baby *Female* *Male*	 *5(45.5)* *6 (50.5)*	 *6 (60)* *4 (40)*	 *7(70)* *4(30)*

Values are expressed as mean (range) for maternal age, gestational age and birthweight and number (percentage) for sex of the baby.

Table 3.2 . Result of analysis of maternal age, gestational age at delivery, birth weight and the sex of the babies' data

ANOVA

Source=Between Groups

	Sum of Squares	df	Mean Square	F	Sig.
maternal age(years)	87.784	2	43.892	1.967	.158
Gestational age at delivery(weeks)	104.557	2	52.278	9.576	.001
Birthweight (grams)	9182698.684	2	4591349.342	11.912	.000
Sex of the baby	.202	2	.101	.382	.686

This is a table of the test statistic result of the between groups analysis of variance. The means of gestational age at delivery and the birth weight are significantly different between the groups (P = 0.001 for GAD and p = 0.000 for birthweight). Means of maternal ages are not significantly different between the groups

Positive Antibody Labelling

Positive immunoreactivity is assumed when immunofluorescence is between 0 and 255 on the grayscale and negative immunoreactivity is when there is no immunofluorescence above background levels i.e. 0 on the grayscales.

Endothelial marker

The antibody specific for von-Willebrand factor has consistently labelled endothelium of the fetal blood vessels (in the core of chorionic villi), the endothelium of maternal blood vessels of the basal plate as well as segments of the lining of the basal plate intervillous border (Fig.3.1, 3.3a, 3.6-3.20, and 3.22-3.27). The labelling pattern of the polyclonal rabbit anti-von Willebrand factor antibody is consistent with that obtained with EN4 antibody, another endothelial marker that is immunoreactive with CD31 antigen or PECAM-1 (Burgio *et al.*, 1994) (Fig.3.3a and b). Although positive control experiments (e.g using blood vessels) were not done, the endothelium of the fetal capillaries in the villi provided an embedded positive control for the basal plate intervillous surface lining endothelium. Negative controls done by omitting the primary antibody or using isotype control at the same dilusion as the anti-von Willebrand antibody showed negative immunofluorescence (Fig. 3.2 and fig. 3.21)

Trophoblast marker

The trophoblast marker used in the experiments was monoclonal mouse anti-pancytokeratin. It was immunoreactive with pancytokeratins in the cytoplasm of trophoblastic epithelium. It was positively immunoreactive with both syncytio- and cyto-trophoblast cells of the chorionic villi, the extravillous cytotrophoblast cells in cell columns and basal plate and segments of the lining of the intervillous border of the basal plate (Fig.3.1, 3.4, 3.6-3.15 and 3.17-3.20). The labelling pattern of the monoclonal pancytokeratin antibody was essentially consistent with that of polyclonal Keratin18 antibody (fig. 3.4 and 3.5). It reacts occasionally with the walls of large maternal blood vessels in the uteroplacental site and connective tissues

of basal plate and chorionic plate (Fig 3.24). There was no significant cross labelling between the endothelial and trophoblast marker antibodies.

In the control experiments, where both primary antibodies were omitted and replaced with 20% fetal calf serum diluted with TBS-T (Fig.3. 2 and 3.21) or with antibodies of the same isotype and concentration as the primary, there was no significant fluorescence above background. This is true for the chorionic plate specimen as well.

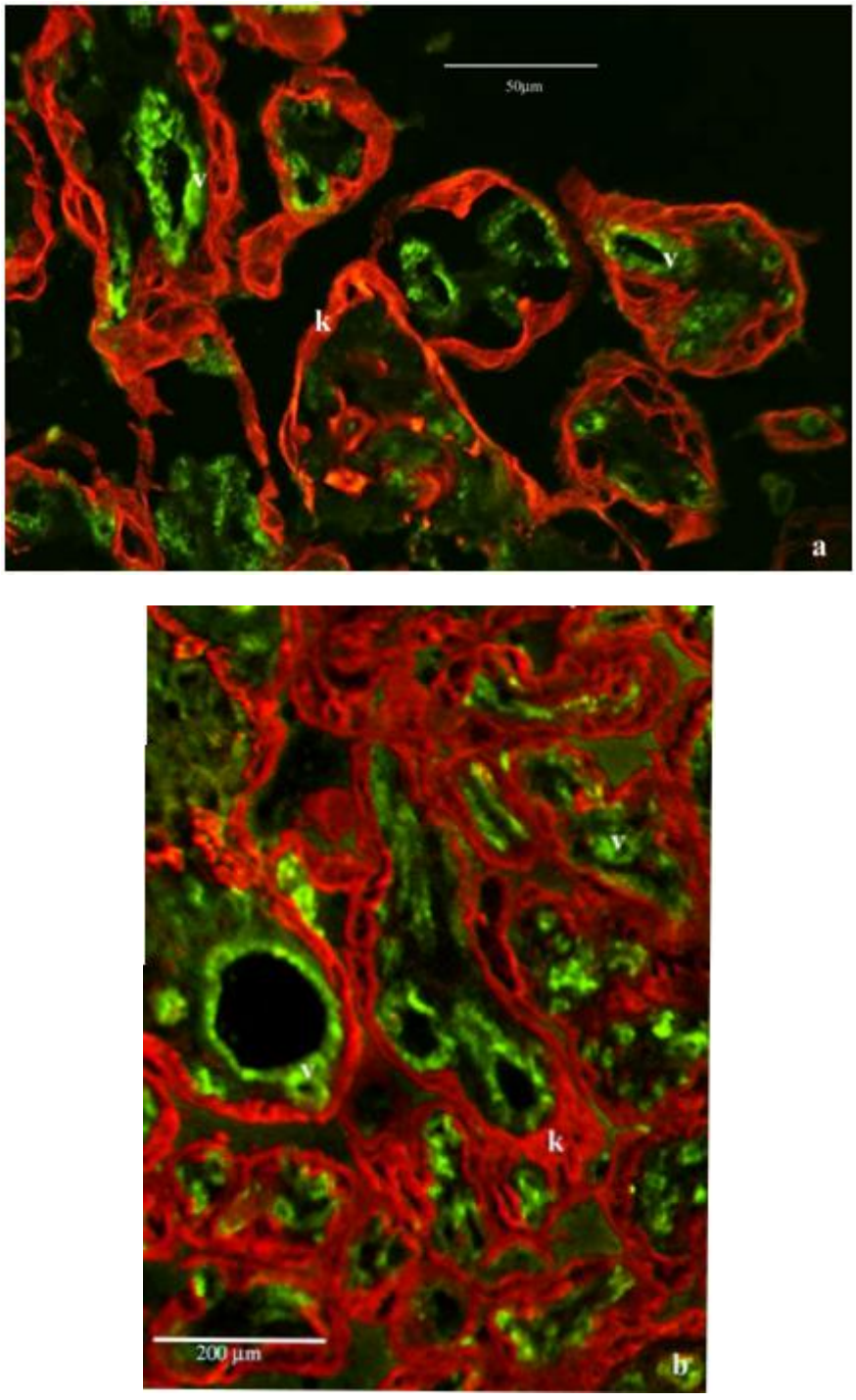


Fig. 3.1 a: This confocal laser scanning micrograph of placenta dual-labelled with anti-pancytokeratin (red) as trophoblast marker and anti-von Willebrand factor (green) as endothelial marker. It shows the pancytokeratin labelling of trophoblastic epithelium (**k**) and von Willebrand factor labelling the endothelium (**v**) of foetal capillaries in the core of the villi. The pattern of the labelling is mutually exclusive and distinctive. The micrograph shows healthy placental tissue. **b**: Shows pre-eclamptic placenta has a similar labelling pattern.

Fig. 3.2 A confocal laser scanning (CLSM) image of the control experiment for fig. 1a. The primary antibodies were omitted and replaced by 20 % fetal calf serum. There is no visible fluorescence above background level.

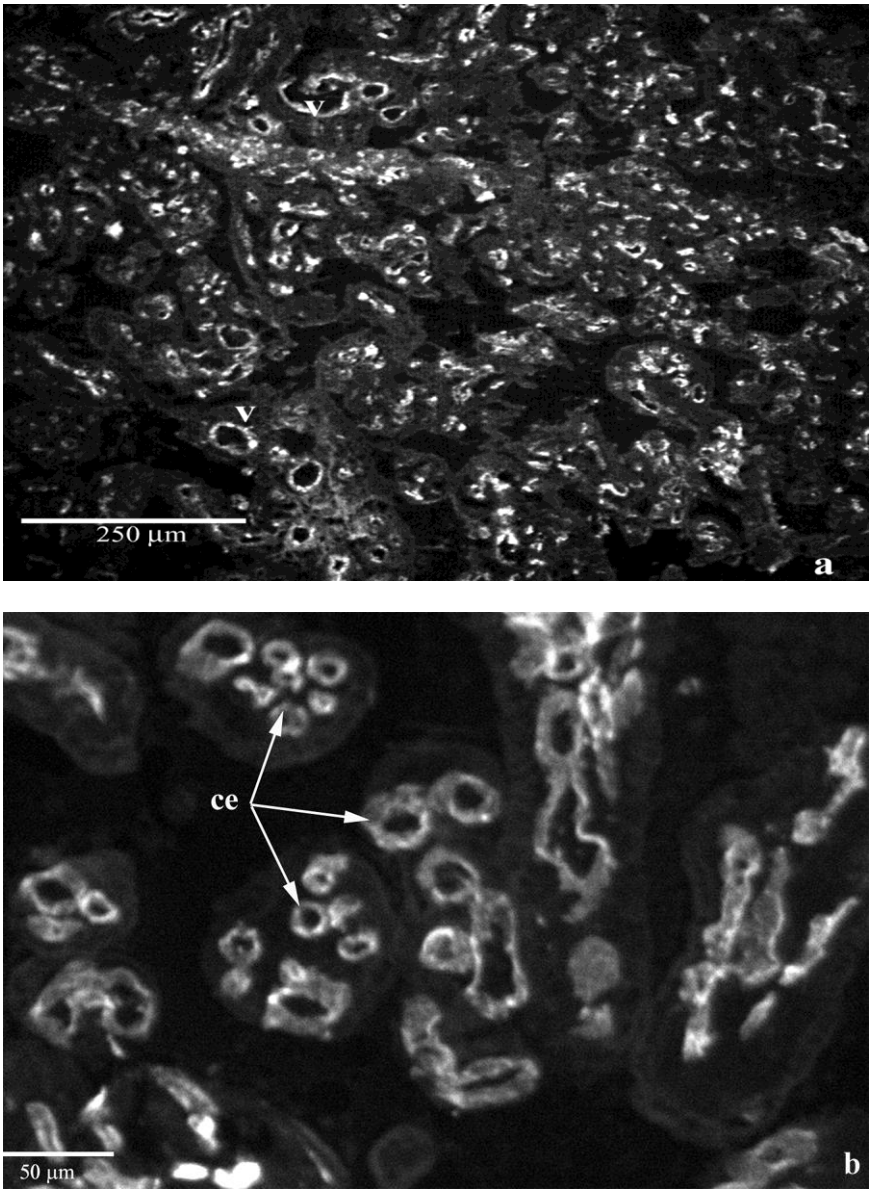


Fig. 3.3 Von Willebrand factor (**a**) and EN4 (**b**) labelled indirect immunofluorescence microscopy images detected using confocal laser scanning microscope showing both antibodies labelling the foetal capillary endothelium (**ce**) within the villi. Though they at different magnifications the pattern of labelling is similar for both antibodies thus substantiating specificity of anti-von Willebrand factor as endothelial marker.

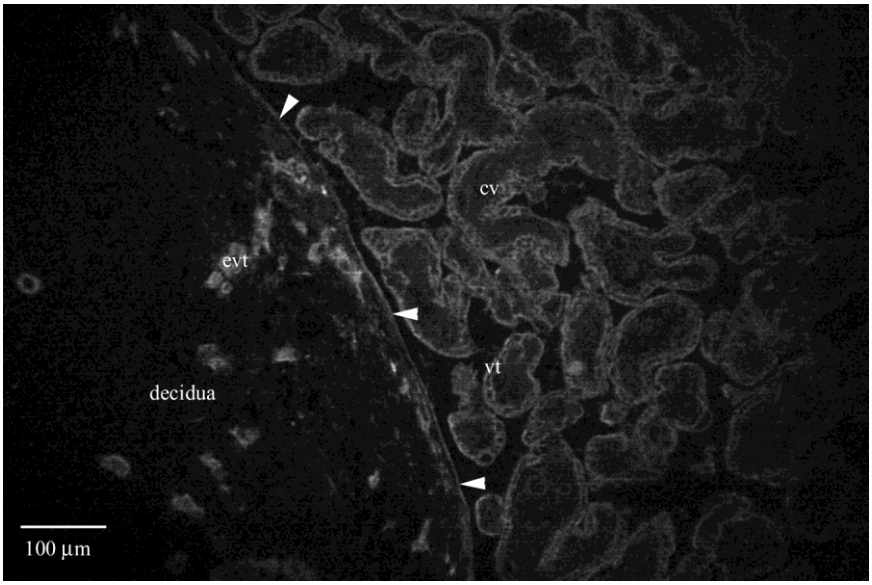


Fig. 3.4 This is a grayscale micrograph of human placental basal plate immunostained with monoclonal mouse anti- pancytokeratin antibody at dilution of 1: 800. On the right is intervillous space with chorionic villi (**cv**) and on the left is the basal plate with decidua and extravillous cytotrophoblast cells (**evt**). The basal plate intervillous surface lining (**arrowheads**) separates the two. This trophoblast marker has clearly outlined villous trophoblast (**vt**) and evts in this image.

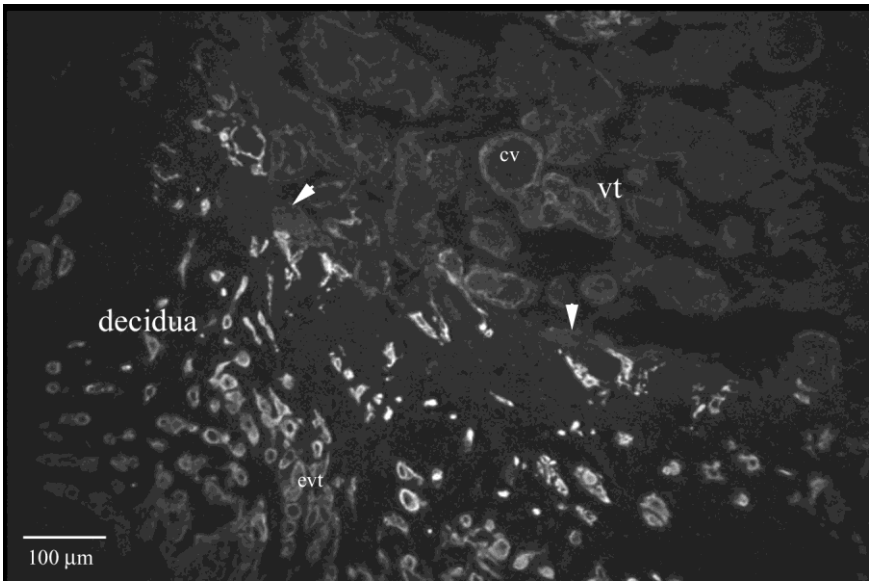


Fig. 3.5 This is a grayscale micrograph of human placental basal plate immunostained with polyclonal rabbit anti-keratin 18 antibody at dilution of 1: 50. On the right is intervillous space with chorionic villi (**cv**) and on the left is the basal plate with decidua and extravillous cytotrophoblast cells (**evt**). The basal plate intervillous surface lining (**arrowheads**) separates the two. This trophoblast marker has clearly outlined villous trophoblast (**vt**) and evts in this image.

Basal plate lining cells

Qualitative Results

The results from the dual-labelled indirect immunofluorescence confirmed the presence of a unicellular layer lining the intervillous border of the basal plate of the placenta (Fig.3.6-3.15 and 3.17-3.20). This lining is made up of cells that express either endothelial or trophoblast antigens suggesting endothelial and trophoblastic composition of the lining (Fig.3.6- 3.15 and 3.17-3.20). The endothelial and trophoblast expressing segments are frequently observed to be continuous end-to-end (Fig. 3.10) but at some points the two cell types overlap each other (Fig. 3.11). The endothelium marker positive cells appear to be widespread within the basal plate lining of the intervillous space border and not limited only to the openings of utero-placental blood vessels (Fig. 3.7, 3.13-3.15).

This mosaic layer is interrupted at some points by gaps which are negative for both immunofluorescence probes (Fig. 3.13). In separate histological experiments in which paraffin embedded placental tissue were stained with Martius scarlet blue (MSB) the results showed that the gaps were filled by fibrin or fibrinoid (Fig. 3.16). Also interrupting the layer at certain places are anchoring villi (Fig. 3.8, 3.11-3.13, 3.16a and 3.17). The allo-epi-endothelial phenomenon has been confirmed. It is possible on the basis of this evidence to conclude that the trophoblast-endothelial mosaic is present over the entire basal plate and not limited to a particular region as it is seen in basal plate specimens sampled from the edge, midway between the edge and middle, and the middle of the placenta. There were no obvious qualitative differences in the labelling pattern of the markers in healthy and pre-eclamptic placentae. Pre-eclamptic placentae appear to have fewer anchoring villi interrupting the lining. The maternal surface of the basal plate (line of separation of the placenta from the uterus) is lined by tissue that is immunoreactive with either trophoblast or endothelium markers but predominantly endothelium (Fig. 3.17).

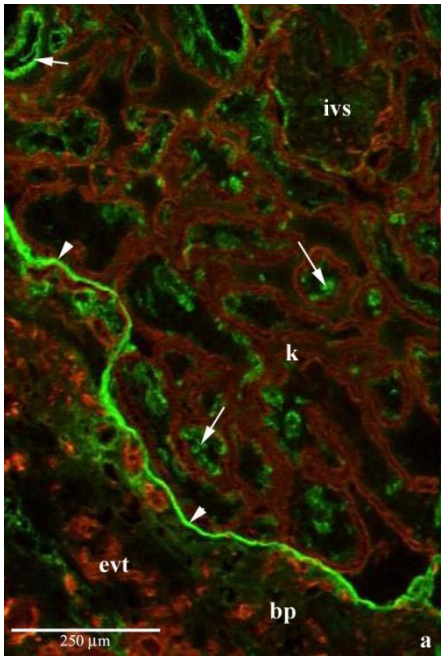


Fig. 3.6 A healthy human placental tissue sample containing a segment of the basal plate (**bp**) and intervillous space (**ivs**). The intervillous space lining (arrowheads) is reactive with endothelial marker (**green**) as is the foetal capillary endothelium (**arrows**). All the **ivs** lining in this image is endothelial. Extravillous trophoblast cells (**evt**) are seen in the basal plate. (**Red** channel = anti-pancytokeratin; **green** = anti-von Willebrand factor; **k** = chorionic villous trophoblast)

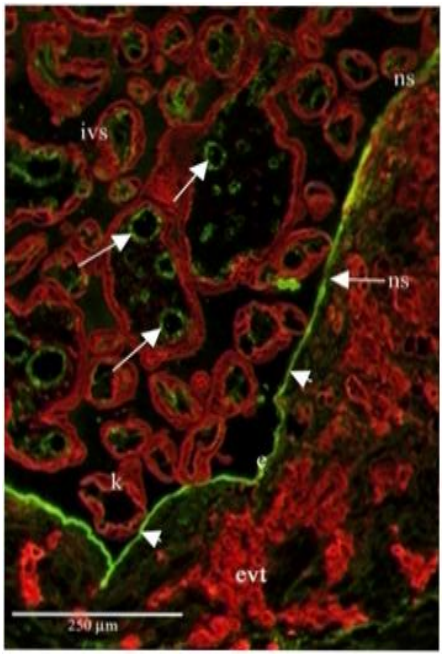


Fig. 3.7. Confocal laser scanning micrograph of pre-eclamptic placental basal plate showing the intervillous space border lining (**arrowheads**) positively immunoreactive with anti-von Willebrand factor (**green**). Foetal capillary endothelium (**arrows**) is similarly positive. The endothelial marker positive cells on the intervillous border cover an extensive length and are characteristically thin as endothelium. Gaps (**ns**) in the layer are negative for the endothelial and trophoblast markers.

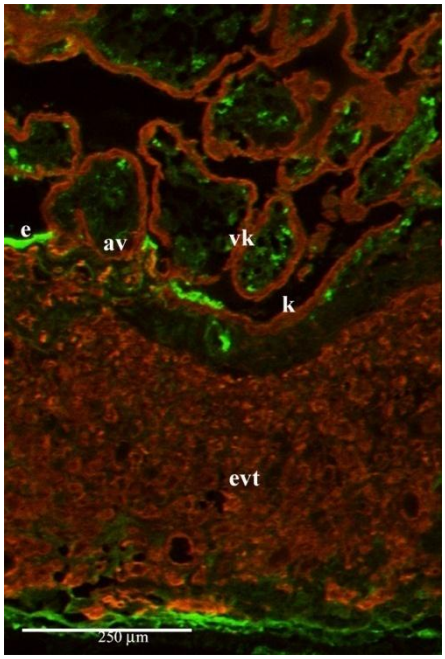


Fig. 3.8 This is an immunofluorescence confocal laser scanning image of the basal plate of a healthy pregnancy placenta showing anti-pancytokeratin antibody (**red**) outlining the basal plate intervillous border lining cells (**k**). Most of the lining cells in this segment are positive for anti-pancytokeratin antibody as is extravillous trophoblast cells (**evt**) and villous trophoblast (**vk**). The lining is interrupted by anchoring villous (**av**) and a short segment of endothelium (**e**).

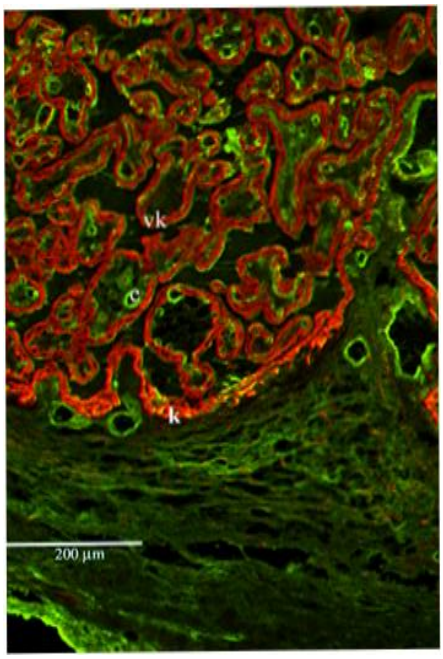


Fig. 3.9 This immunofluorescence confocal scanning image of the basal plate of a pre-eclamptic pregnancy placenta showing anti-pancytokeratin antibody (**red**) outlining the basal plate intervillous border lining cells (**k**). Most of the lining cells in this segment are positive for pancytokeratin antibody as are villous trophoblast (**vk**). (e = foetal capillary endothelium). Connective tissues in the basal plate appear to be picking von- Willebrand factor but the immunofluorescence intensity is less than that of foetal capillary endothelium.

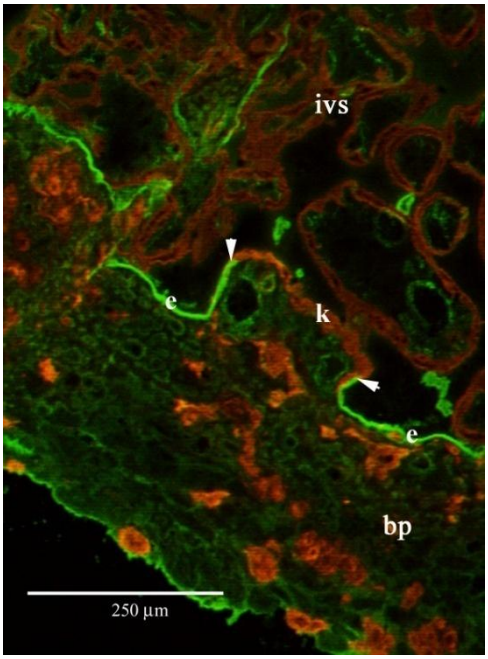


Fig. 3.10. This is a dual-labelled imunofluorescence confocal laser scanning image of the basal plate of a healthy pregnancy placenta showing the intervillous surface lining of the basal plate consisting of a mosaic of endothelium (**e**) and trophoblast (**k**). The endothelium, of endodermal origin, is seen making cell-to-cell contact (**arrowheads**) with trophoblast of ectodermal origin. Together these two cells types form the lining layer. (**bp**= basal plate; **ivs**= intervillous space).

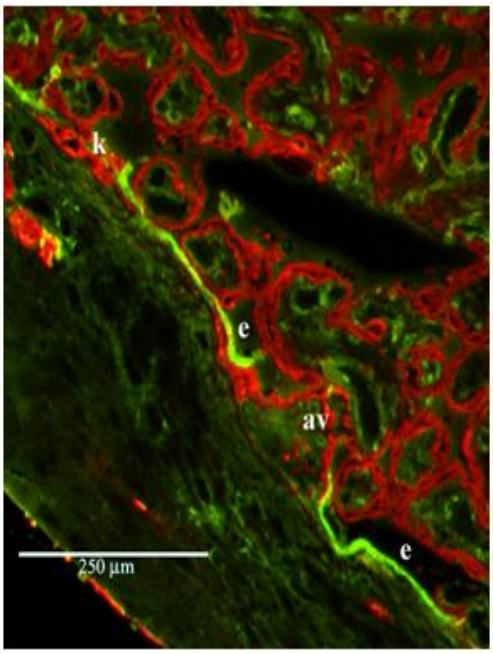


Fig. 3.11. A micrograph of a pre-eclamptic pregnancy placenta basal plate showing intervillous border lining consisting of endothelium (**e**) and trophoblast (**k**). These two cells make contact with each other and they appear to overlap each other at some points. Anchoring villous (**av**) is seen interrupting the unicellular layer. AV represents a point where extravillous cells in the cell column make contact with maternal tissues in the decidua.

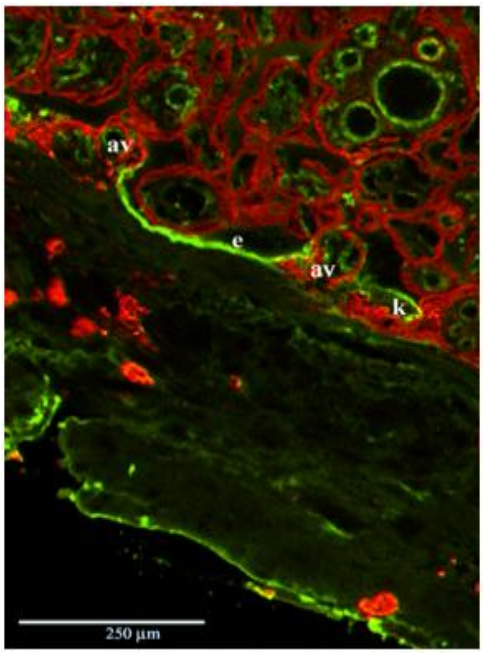


Fig. 3.12. A micrograph of a pre-eclamptic pregnancy placenta basal plate,dual-labelled with anti-von Willebrand factor (**green**) and anti-pancytokeratin (**red**) showing the intervillous space border lining layer being interrupted by anchoring villi (**av**). (**e** = endothelium; **k**= trophoblast).

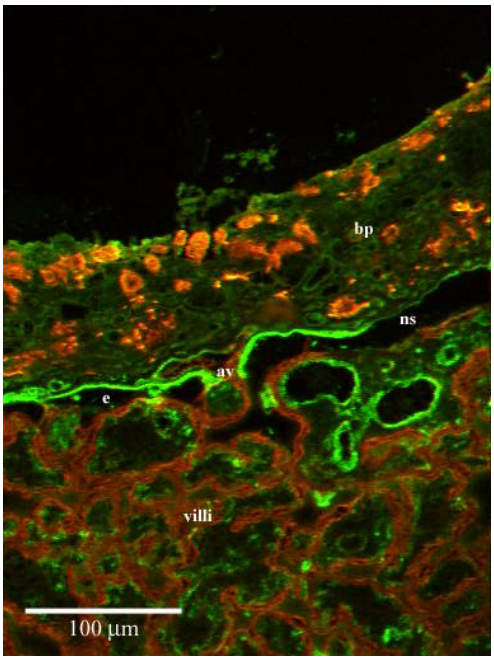


Fig. 3.13 Confocal laser scanning image of a healthy placental basal plate dual-labelled with anti-pancytokeratin (**red**) and anti-von Willebrand factor (**green**). The intervillous space border is lined in part by endothelium (**e**). An anchoring villous (**av**) is seen making contact with the basal plate (**bp**). Part of the lining is not reactive with both antibodies (**ns**) and it has been shown in histological preparations to correspond with areas of fibrin/ fibrinoid deposition

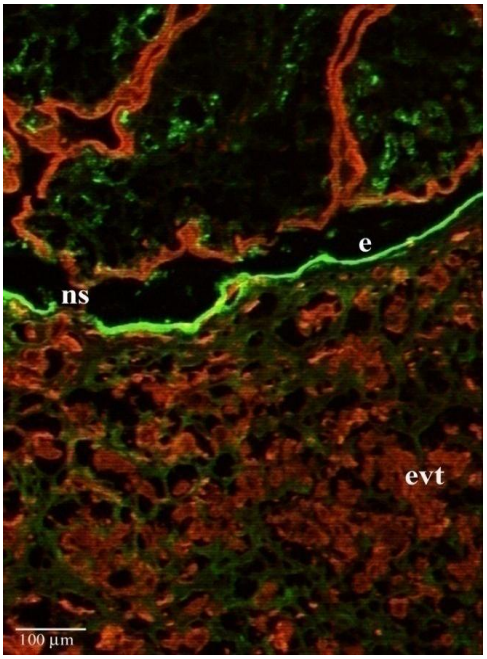


Fig. 3.14. This is an image of a healthy pregnancy placental basal plate showing part of the intervillous surface to be reactive with the endothelial marker **(e**) and part which is not reactive with both endothelial and trophoblast marker antibodies (**ns**). These gaps are thought to represent areas of fibrin/fibrinoid deposition. **evt** = extravillous trophoblast.

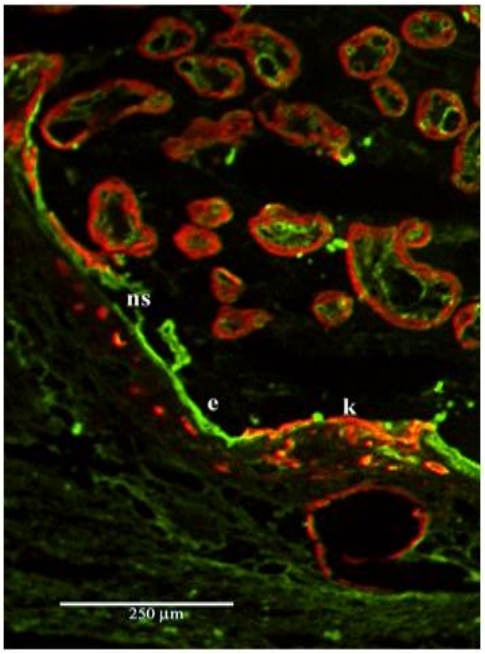


Fig. 3.15. A confocal laser scanning image of placenta basal plate, showing the intervillous surface lined by trophoblast (**k**) and endothelium (**e**). The layer is interrupted by a segment that is negative for both trophoblast and endothelial markers (**ns**). These gaps correspond with areas of fibrin deposition in histological preparations.

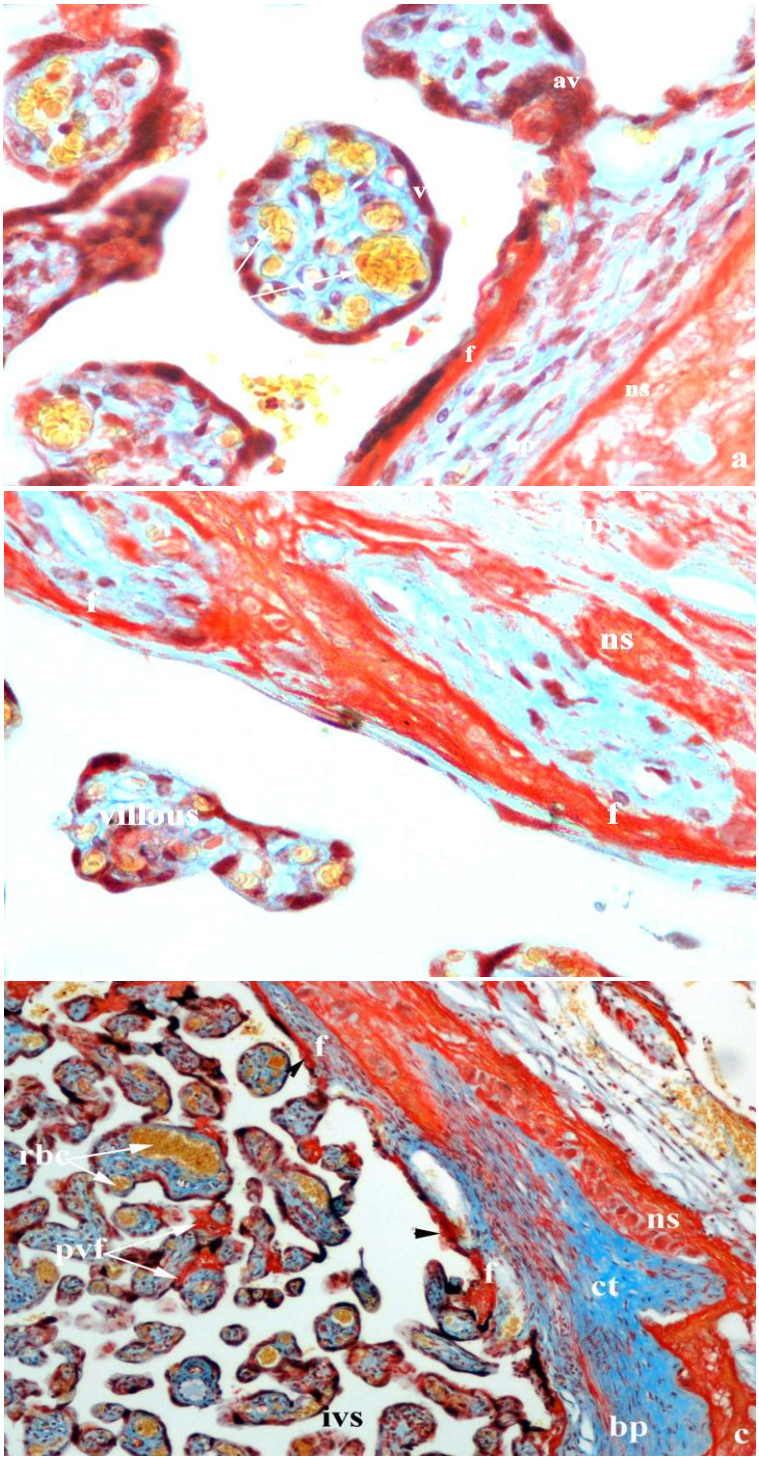


Fig. 3.16. **a,b,c**. Histological images of the placental basal plate stained with Martius Scarlet Blue (MSB) showing a thick band of fibrin (**f**) beneath the cellular layer lining the intervillous surface. This fibrin layer replaces the cellular layer at places where the cellular layer is absent (**arrowheads**). **vt** = Villous syncytiotrophoblast; **bp** = basal plate; **ivs** = intervillous space; **e** = endothelial cell; **av** = anchoring villous, **pvf** = perivillous fibrinoid, **ct** = connective tissue, **ns** = Nitabuch's stria, **rbc** = red blood cells.

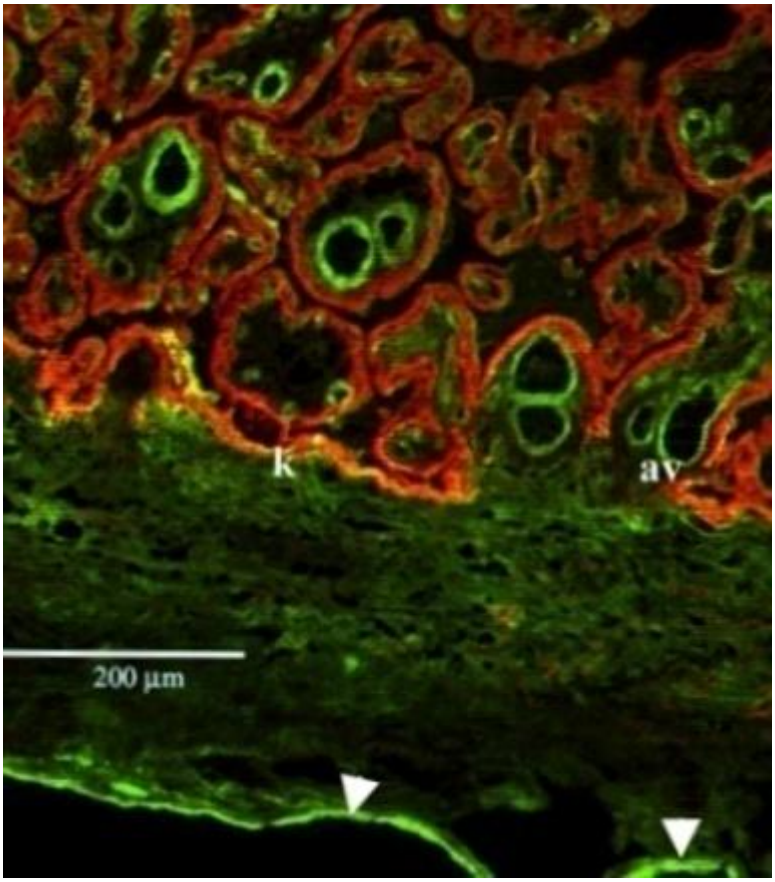


Fig. 3.17. This is an image from pre-eclamptic placental basal plate. Dual indirect immunofluorescence shows that von Willebrand factor-positive cells are lining the maternal surface (abscission layer) of the basal plate (**arrowheads**). (k = pancytokeratin positive cells; av = anchoring villous).

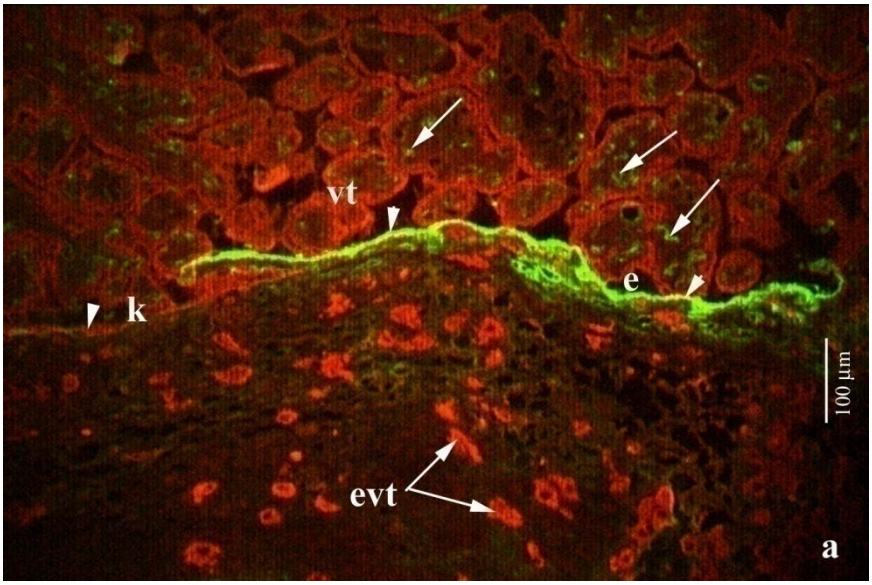


Fig. 3.18. This is a human placenta basal plate sampled from the edge of the placenta and dual-labelled with anti-pancytokeratin (**red**) and anti-von Willebrand factor (green). The intervillous surface of the basal plate (**arrowheads**) separates maternal tissues in the deciduas (**below**) from the villi in the intervillous space. A segment of the lining of the intervillous surface of the basal plate (**e**) is outlined by endothelial marker and part (**k**) is also outlined by trophoblast marker. Arrows = foetal capillary endothelium; **vt** = villous trophoblast; **evt** = extravillous trophoblast

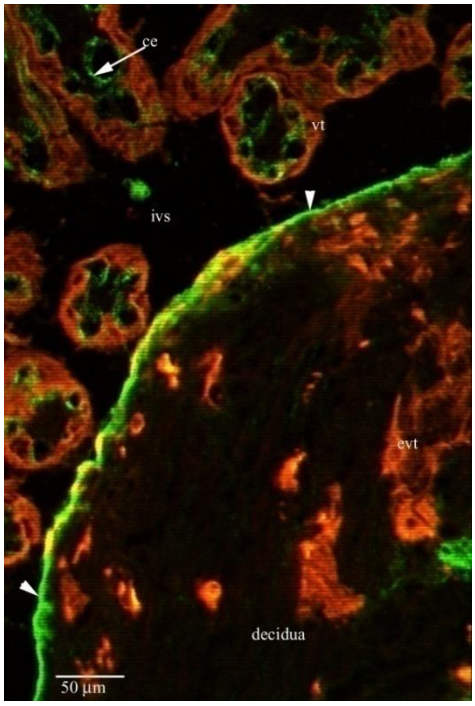


Fig. 3.19. This micrograph shows a human placenta basal plate sampled from midway between the edge and the centre of the placenta and dual-labelled with anti-pancytokeratin (**red**) and anti-von Willebrand factor (green). The intervillous surface of the basal plate (**arrowheads**) is outlined by endothelial marker. Arrows = foetal capillary endothelium; **vt** = villous trophoblast; **evt** = extravillous trophoblast

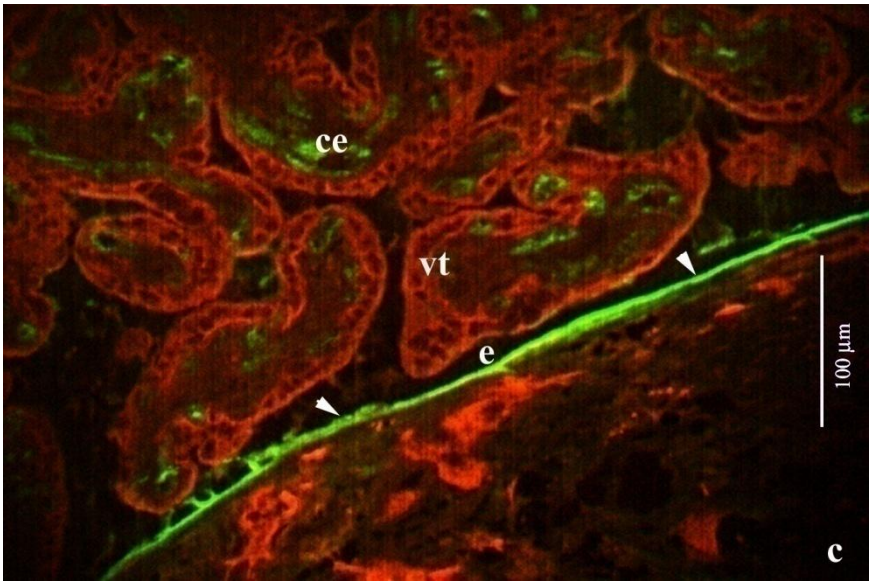


Fig. 3.20. This is human placental basal plate sampled from the centre of the placenta and dual-labelled with anti-pancytokeratin (**red**) and anti-von Willebrand factor (**green**). The intervillous surface of the basal plate (**arrowheads**) separates maternal tissues of the deciduas (below) from the chorionic villi in the intervillous space above. A segment of the lining of the intervillous surface of the basal plate (**e**) is outlined by endothelial marker. **ce** = foetal capillary endothelium; **vt** = villous trophoblast; **evt** = extravillous trophoblast.

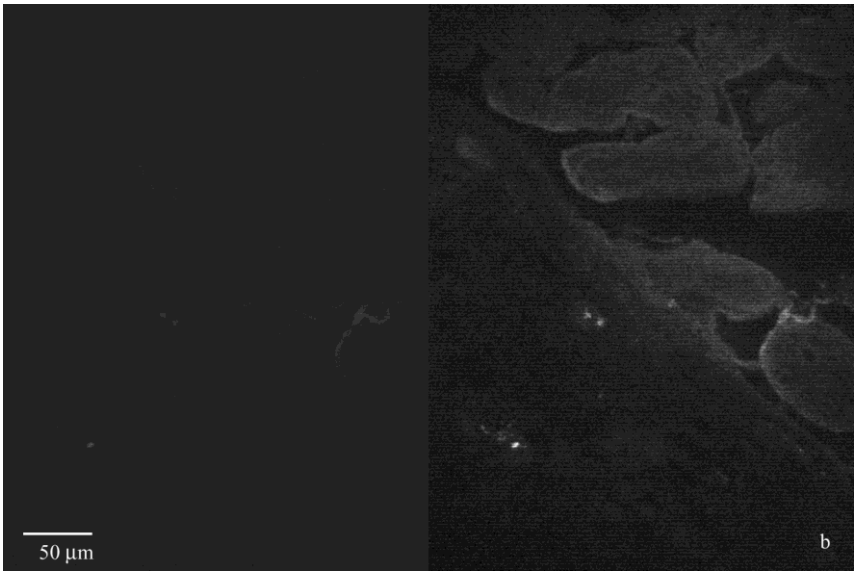


Fig. 3.21. This is CLSM image of human placenta from the same placental sample as fig.3.20 above In this control experiment IgG1 isotype of the monoclonal mouse anti-pancytokeratin (1 : 800 dilution) and IgG isotype of polyclonal anti-vWF (1 : 400 dilution) were used to replace the specific primary antibodies. There is no immunofluorescence above background levels

Quantitative results

Comparisons of proportions within the patients groups

Table 3.5 and Tables 5.11-5.14 show the means and median of the data for the three placental groups. Measurements of the basal plate lining components of the images of the control placentae showed that the mean length of endothelium and trophoblast are 62.83% and 27.64% respectively (Table 3.5, 5.11 and 5.12). The fibrin/fibrinoid and anchoring villi form 5.93% and 3.64% respectively of the basal plate lining (Table 3.5, 5.13 and 5.14). The mean proportions of endothelium and trophoblast for mild pre-eclamptic placentae were 69.79% and 20.83% respectively (Table 3.5, 5.11 and 5.12). Fibrin and anchoring villi make up 7.44% and 1.95% of the lining of mild pre-eclamptic placentae respectively (Table 3.5, 5.13 and 5.14). For the severe pre-eclamptic placentae, the mean values for endothelium, trophoblast, fibrin and anchoring villi are 51.23%, 35.42% 11.12% and 2.23% respectively (Table 3.5 and 5.11-5.14). Clearly therefore, endothelium forms a larger proportion of the basal plate intervillous space lining in all three patients groups. There was no observed regional variation in the length percent data for the specimens sampled from the four different areas of each placeneta (refer to basal plate sampling).

The Kolmogorov-Smirnov (K-S) and Shapiro-Wilk's (S-W) tests of normality for the length percent of the basal plate component data for each group shows the test is not significant ($p > 0.05$) for all the components in all the groups except the length percent fibrin data for the control. The data for the endothelial, trophoblast and anchoring villi are normally distributed. The fibrin data is normally distributed for the mild and severe pre-eclamptics but is asymmetrically distributed in the control patient group (Tables 3.3, 3.4 and 5.15). The statistical test used was therefore ANOVA. The K-S and S-W tests of normality for the transformed data (NS-AV, AV to NS ratio and END to TPB ratio) for each group revealed that the K-S test is not significant for all the data in all the groups. The S-W test is not significant for the NS-AV and AV to NS ratio in all the groups. It is however significant for

END to TPB ratio in all the groups. Hence the distribution of the NS-AV and AV to NS ratio data fit normal distribution. The END to TPB ratio data is not normally distributed (Refer appendix 5, Table 5.20).

Comparison of the mean proportions of the various components of the basal plate lining within each of the three patient groups showed that the mean length proportion of endothelium, trophoblast, anchoring villi and fibrin differ significantly within each patients group (ANOVA, p=0.000 for each group). Multiple comparisons between the lining components has revealed that in placentas from healthy controls and those from mild pre-eclamptic pregnancies, there is a larger proportion occupied by endothelium than trophoblast, fibrin/fibrinoid and anchoring villi (ANOVA, p = 0.000 for control and mild pre-eclampsia; posthoc test, p = 0.000 for trophoblast, fibrin/fibrinoid and anchoring villi in both patient groups). Within the severe pre-eclamptic patient group however, there are no significant differences between the mean length proportions of endothelium and trophoblast (posthoc test, p = 0.172). The mean length percent of fibrin/fibrinoid and anchoring villi are not different within the three groups of patients (post hoc, p = 1. 00).

There is a significantly positive correlation between the level of maternal systolic BP and length percent of basal plate IVS fibrin (Pearson's correlation coefficience r = 0.36, p = 0.45) (refer to table 3.5 and fig. 3.22). There was positive correlation between length of fibrin and highest diastolic blood pressure but it was not statistically significant(r = 0.308, p = 0.086). There appears to be a negative correlation between length percent of endothelial cells and maternal SBP and DBP but the correlation is not significant (r = -0.063, p = 0.734 for SBP and r = -0.68, p= 0.712 for DBP). There was no correlation between length of fibrin and GAD (r = 0.033, p = 0.858) and length of endothelium and GAD (r = 0.011, p = 0.96). Birth weight appears to be positively correlated with length percent of endothelium but the

correlation is not significant (r = 0.18, p = 0.32). Length percent of the basal plate IVS fibrin appears to have no effect on the birth weight (r = 0.006, p = 0.953).

Table 3.3. Test=Kolmogorov-Smirnov

Tests of Normality

Dependent Variables	Patient group	Statistics		
		Statistic	Df	Sig.
Length percent endothelium	Healthy Control	.125	11	.200*
	Mild pre-eclamptic	.155	10	.200*
	Severe pre-eclamptic	.195	11	.200*
Length percent trophoblast	Healthy Control	.149	11	.200*
	Mild pre-eclamptic	.181	10	.200*
	Severe pre-eclamptic	.176	11	.200*
Length percent anchoring villi	Healthy Control	.191	11	.200*
	Mild pre-eclamptic	.220	10	.186
	Severe pre-eclamptic	.254	11	.046
Length percent fibrin/fibrinoid	Healthy Control	.262	11	.033
	Mild pre-eclamptic	.181	10	.200*
	Severe pre-eclamptic	.136	11	.200*

*. This is the lower bound of the true significance.

Kolmogorov-Smirnov test for normal distribution for length percent of endothelium, trophoblast, fibrin/fibrinoid and anchoring villi for the 3 placental groups. The test shows that the data is normally distributed as p>0.05(sig. column) thus, the null hypothesis that the data is drawn from a population other than a normally distributed population is rejected.

Table 3.4 : Test=Shapiro-Wilk

Tests of Normality

Dependent Variables	Patient group	Statistics		
		Statistic	Df	Sig.
Length percent endothelium	Healthy Control	.976	11	.939
	Mild pre-eclamptic	.918	10	.338
	Severe pre-eclamptic	.861	11	.060
Length percent trophoblast	Healthy Control	.942	11	.549
	Mild pre-eclamptic	.943	10	.588
	Severe pre-eclamptic	.908	11	.228
Length percent anchoring villi	Healthy Control	.924	11	.350
	Mild pre-eclamptic	.865	10	.088
	Severe pre-eclamptic	.864	11	.065
Length percent fibrin/fibrinoid	Healthy Control	.822	11	.018*
	Mild pre-eclamptic	.948	10	.645
	Severe pre-eclamptic	.960	11	.770

Shapiro-Wilk test for normal distribution for length percent of endothelium, trophoblast, fibrin/fibrinoid and anchoring villi for 3 placental groups. The test shows that the data is normally distributed in all except length percent fibrin in the control group as $p>0.05$(sig. column). The fibrin is significant as p value (Asteric) <0.05.

Table 3.5 Correlation betweenLength percent of fibrin and highest systolic blood pressure

Correlations

		Length percent fibrin/fibrinoid	Highest systolic Blood pressure in mmHg
Length percent fibrin/fibrinoid	Pearson Correlation	1.000	.355*
	Sig. (2-tailed)		.046
	N	32	32
Highest systolic Blood pressure in mmHg	Pearson Correlation	.355*	1.000
	Sig. (2-tailed)	.046*	
	N	32	32

*. Correlation is significant at the 0.05 level (2-tailed).

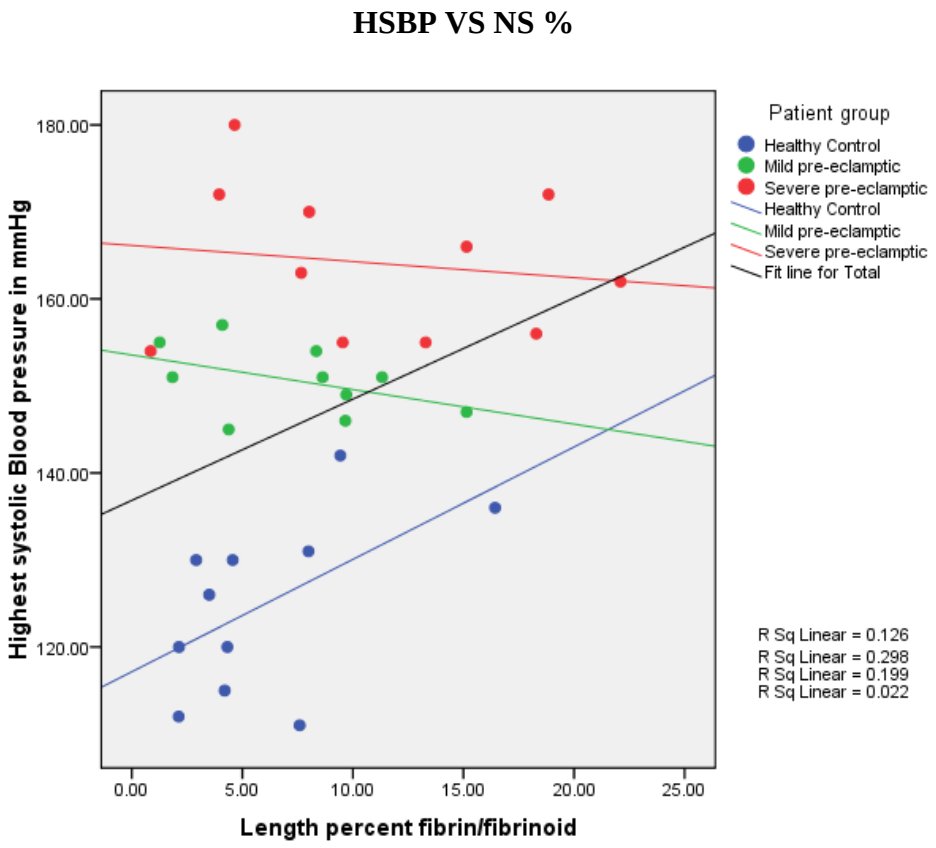


Fig. 3.22 A plot of highest SBP against length percent of fibrin. There is overall positive correlation between HSBP and length of fibrin.

Comparison between the patient groups

Analysis of confocal laser scanning micrographs of placentae from the three patient groups shows that the mean length proportions of the basal plate lining that is covered by fibrin/fibrinoid is larger in pre-eclamptic groups compared to healthy controls but length percent of anchoring villi decreases in the pre-eclamptic groups. On their own the means of length percent of fibrin/fibrinoid (NS) and anchoring villi (AV) are not significantly different between the patients groups ($p = 0.082$ and $p = 0.086$ respectively) (Tables 3.6 and 5.16). The mean of the ratios of the length percent of anchoring villous to length percent of fibrin/fibrinoid is however significantly different between the patient groups (ANOVA, $p = 0.010$) (Tables3.6 and 5.22). Poshoc test revealed that severe preeclampsia has a significantly lower mean of anchoring villi to fibrin ratio than healthy control (Posthoc, $p = 0.009$) but there is no significant difference when severe and mild pre-eclampsia placentae are compared (posthoc, $p = 0.628$) (Tables 3.6 and 5.23). Similarly, combining the data of the two components by deducting the length percent of anchoring villi from length percent of fibrin/fibrinoid generated a data (NS-AV), the mean of which is significantly different between the patients groups (ANOVA, $p = 0.033$) (Table 3.6). Furthermore, severe pre-eclamptic placentae have a significantly larger mean length percent of NS - AV than healthy control placentae (ANOVA posthoc, $p = 0.025$) but there is no difference in the mean of NS - AV between severe and mild pre-eclamptic (ANOVA posthoc, $p = 0.357$) on one hand, and mild pre-eclamptic and healthy control (ANOVA posthoc, $p = 0.399$) on the other (Tables 3.6, 5.23 and 5.24).

The mean length proportion of fibrin/fibrinoid segments is higher in the severe pre-clamptic placentae than in mild pre-eclamptic and the healthy controls but the difference is not significant (ANOVA, $p = 0.082$) (Table 5.16).

Severe pre-eclamptic placentas are associated with a trend of lower mean length proportion of endothelial cells (51.23%) and larger mean length proportion of pan-cytokeratin positive trophoblast cells (35.42%) compared to mild pre-eclamptic (mean of 69.79% and 20.83% respectively)) and healthy control (62.83% and 27.64 %)) but this decrease in endothelial lining in severe pre-eclamptics is not statistically significant (ANOVA, P = 0.107) (Tables 3.6 and 5.16). Also comparison of the mean length percent of trophoblast between the patients groups revealed that there are no significant differences (ANOVA p = 0.131) (Tables 3.6 and 5.16). Furthermore, there are no significant differences between the groups when the median of endothelium to trophoblast ratios are compared (Kruskal Wallis, p = 0.173) (Tables 3.6 and 5.25). Placentas from pre-eclamptic patients with associated foetal growth restriction in pregnancy show a tendency towards a significantly reduced length percent of endothelium and increase proportion of trophoblast cell layer. Owing to the small number of patients with this classification, this tendency could not be tested statistically.

Table 3.6. Summary of statistics Results.

Component	p	Healthy control	pcvm	Mild PE	pmvs	Severe PE	psvc
END	0.107	62.83(63.49)	0.946	69.79(70.97)	0.23	51.23(60.94)	0.356
TPB	0.131	27.64(28.71)	0.829	20.83(18.18)	0.219	35.42(34.84)	0.491
AV	0.086	3.64(3.69)	0.198	1.95(1.29)	0.99	2.23(1.83)	0.232
NS	0.082	5.93(4.32)	0.463	7.44(8.49)	0.578	11.12	0.078
NS-AV	0.033*	2.28(-0.57)	0.399	5.49 (5.32)	0.357	8.89(7.36)	0.025*
AV/NS	0.010*	1.04(1.13)	0.089	0.46(0.26)	0.628	0.22(0.23)	0.009*
END/TPB	0.173	3.89(2.21)		5.80(4.03)		2.59(1.75)	

Values represent mean length percent, (median length percent) and p values. P = p value comparing the three groups, pcvm = p value of control compared to mild pre-eclampsia; pmvs = p value of mild PE compared to severe PE; psvc = p value of severe PE compared to control; END = endothelium, TPB = trophoblast cells; AV = anchoring villous; NS = fibrin; PE= pre-eclampsia. * = significant at p = 0.05.

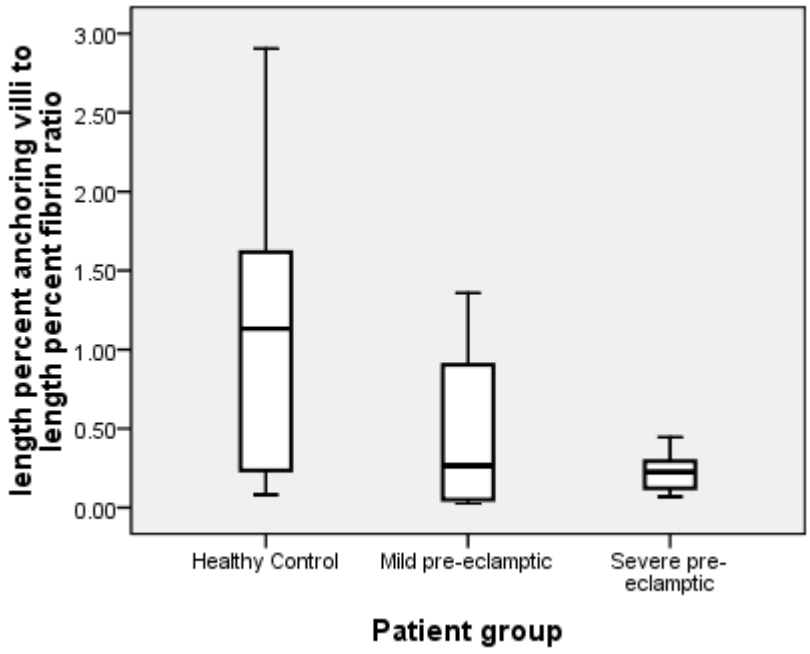


Fig. 3.23. This boxplot show the distribution of the ratio of length percent of anchoring to length percent of fibrin for the patient group. It shows the medians (solid lines in the box) and the end of the whiskers represents the minimum and the maximum values in the data. The median is lower in the pre-eclamptic groups

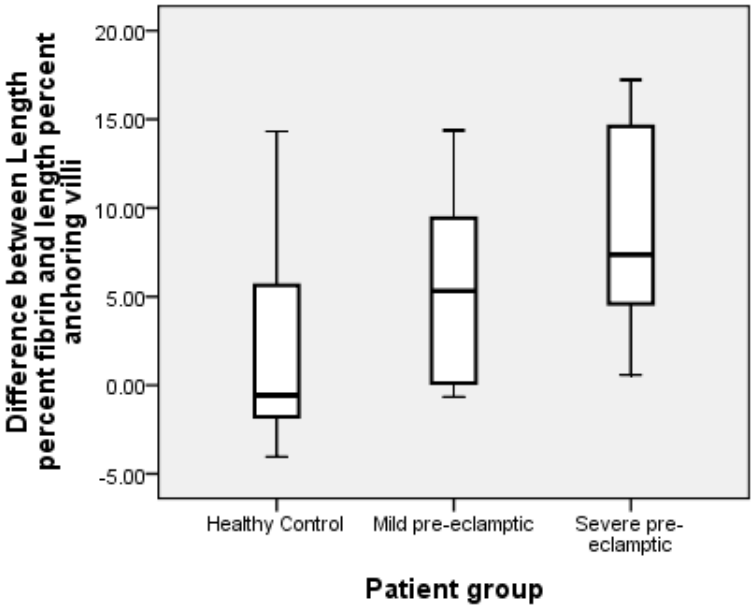


Fig. 3.24. This is the boxplot of the difference between length percent of fibrin and length percent of anchoring villous (NS-AV) for the three patients groups. It shows the medians (solid lines in the box) increase with severity of pre-eclampsia and the whiskers show the spread.

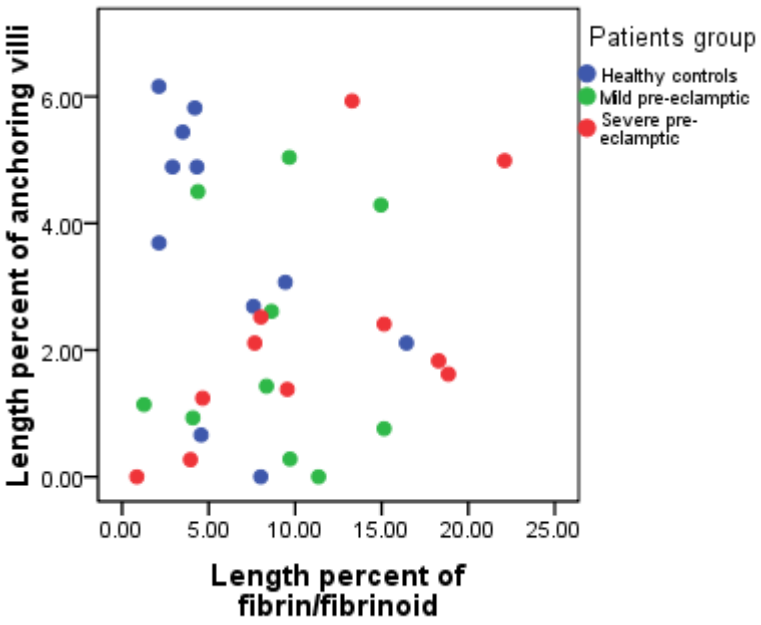


Fig. 3.25.Scatter plot of length percent of anchoring villous versus length percent of fibrin for the three patient groups.

Chorionic plate results

Qualitative results

The dual-labelled immunofluorescence confocal laser scanning microscopy images of chorionic plate samples (Fig. 3.26-3.31) reveal specific anti-keratin and anti-von Willebrand factor indirect immunofluorescence labelling of the cytoplasm of trophoblast and endothelial cells respectively, lining the intervillous surface of the chorionic plate. The lining of the chorionic plate seen here appears to be a monolayer similar to the one described for the basal plate above. The monolayer forms a continuous lining and does conform to the allo-epi-endothelial concept.

The endothelial labelling of the intervillous border of the chorionic plate is seen in samples from the edge of the placenta, midway between the edge and central cord insertion point, and

the centre of the placenta (Fig. 3.29). The endothelial labelling pattern is not related to the positions of chorionic blood vessels. Unlike the basal plate, the endothelial segments of the intervillous space border of the chorionic plate are short and less frequent. The surfaces of these endothelial marker positive layers are less smooth at even lower magnification as they show some projections (Fig. 3.28 and 3.37).

A substantial length of the lining is negative for both trophoblast and endothelial markers. These gaps appear to correspond with areas of fibrin/oid deposits in histological preparations (Fig. 3.32).

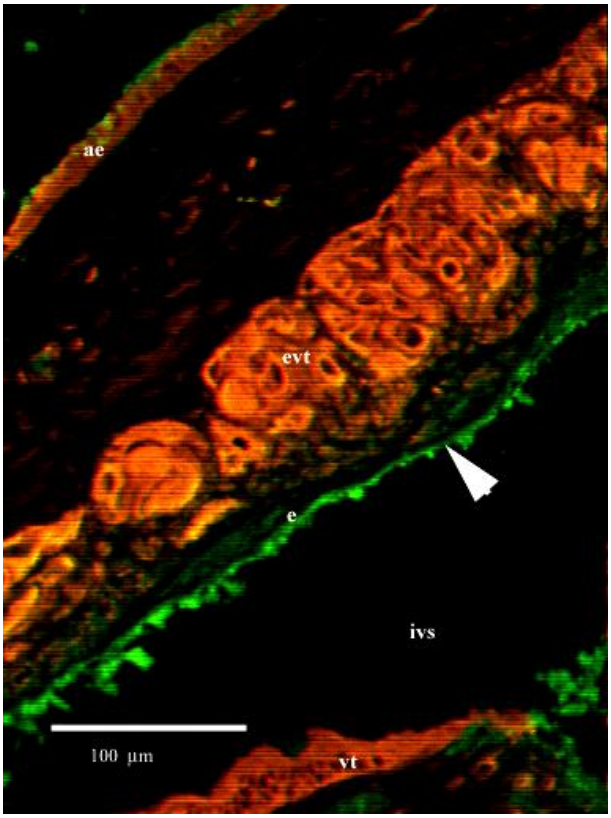


Fig. 3.26: This is a confocal laser scanning micrograph of placental chorionic plate dual-labelled with anti-von Willebrand factor (green) as endothelial marker and anti-pancytokeratin (red) as trophoblast marker antibodies. It shows keratin containing epithelial tissues like amniotic epithelium (ae), villous trophoblast (vt) and extravillous trophoblast (evt) outlined by the trophoblast marker while the intervillous surface of the chorionic plate (arrowhead)is reactive with endothelial marker. ivs = intervillous space; vt = villous trophoblast

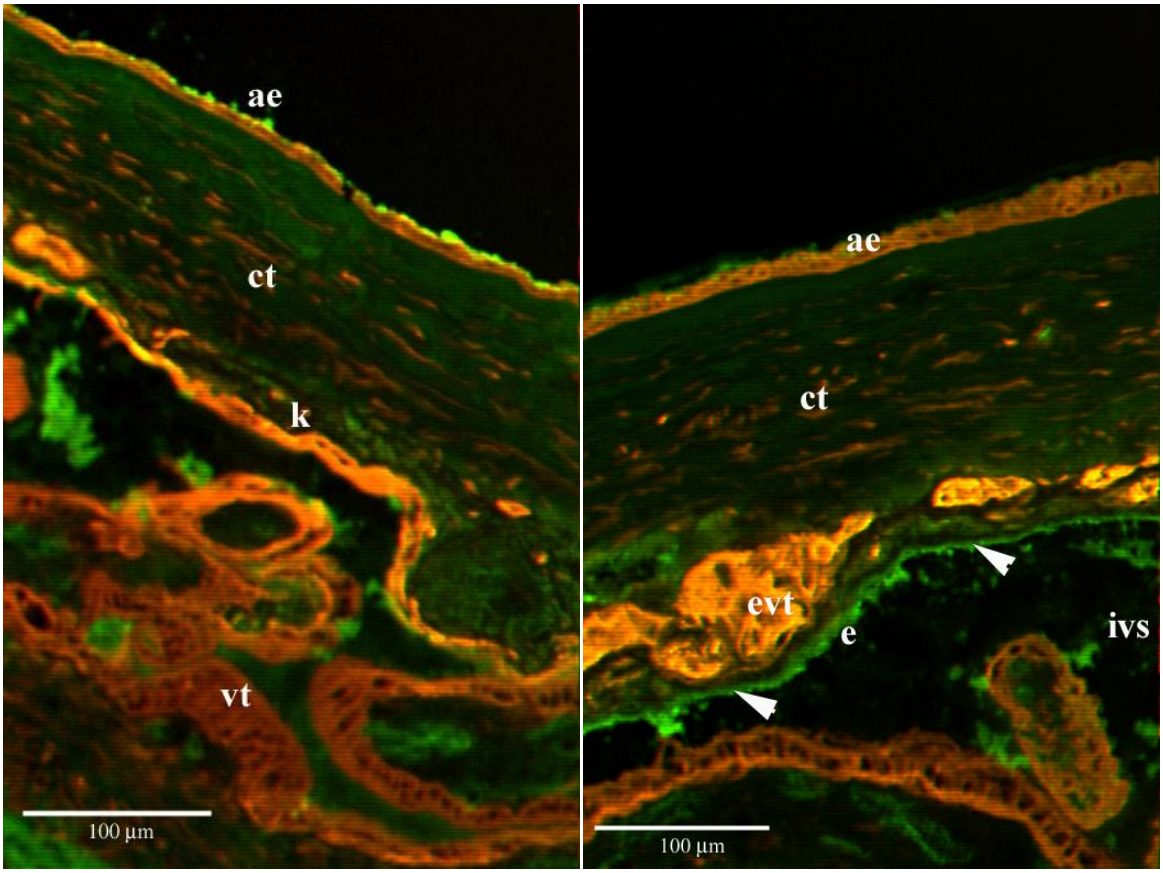
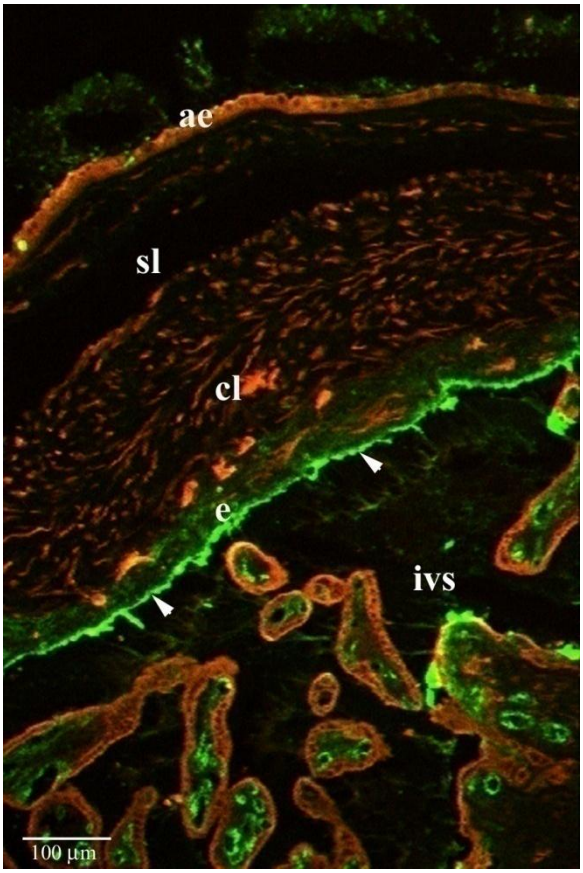
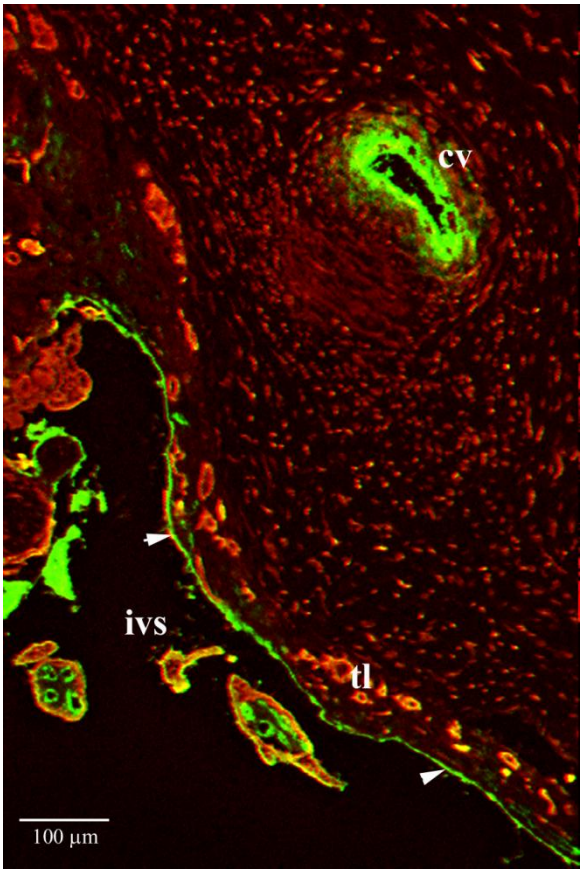


Fig. 3.27: These are immunofluorescence images of chorionic plate from a healthy pregnancy dual-labelled with endothelial marker (**green**) and trophoblast marker (red). The image on the left side shows the chorionic plate intervillous surface (**k**) outlined by the trophoblast marker. The intervillous surface on the right side (arrowheads) however is immunoreactive with endothelial marker. Amniotic epithelium (**ae**), connective tissue in the chorionic plate (**ct**), extravillous trophoblast (**evt**) and villous trophoblast (**vt**) are also immunoreactive with the trophoblast marker.



a



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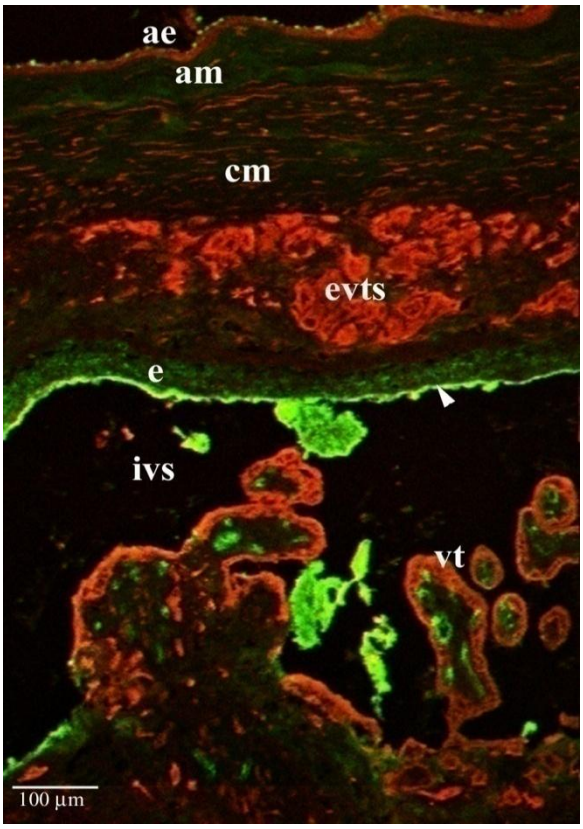

c

Fig. 3.28 a, b and c: These are confocal laser scanning micrographs of chorionic plate from one normotensive pregnancy placenta dual-labelled with endothelial marker (green) and trophoblast marker (red). **a** is sampled from the edge, **b** was sampled from midway between the edge of the placenta and the central cord insertion and **c** was sampled from the centre of the placenta. The intervillous surfaces in all three images (arrowheads) are outlined by the endothelial marker. The trophoblast marker is reactive with keratin expressing tissues of epithelial origin, namely amniotic epithelium (**ae**), extravillous trophoblast (**evt**) and villous trophoblast (**vt**). Other layers visible are amniotic mesoderm (am), spongy layer (**sl**), chorionic mesoderm (cm) and trophoblast layer (**tl**). (e = endothelium; cl = connective tissue layer; **cv** = chorionic vessel; **ivs** = intervillous space)

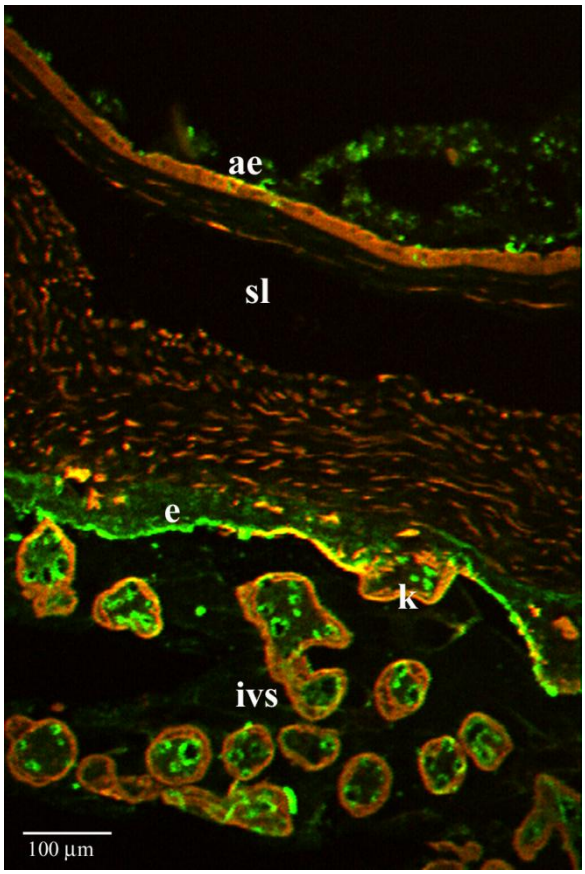

Fig. 3.29: The intervillous surface of chorionic plate from a normotensive pregnancy placenta is seen here consisting of segment that is endothelial marker (anti-von Willebrand factor) positive (e) and another that is trophoblast marker (anti-pancytokeratin) positive (k). The two cell types make cell-cell contact with each other although they are from different embryonic origin. Foetal capillary endothelium is also outlined by the endothelial maker while amniotic epithelium (ae), connective tissue and villous trophoblast are trophoblast marker positive. Sl represents the line of separation between the amnion and the chorion.

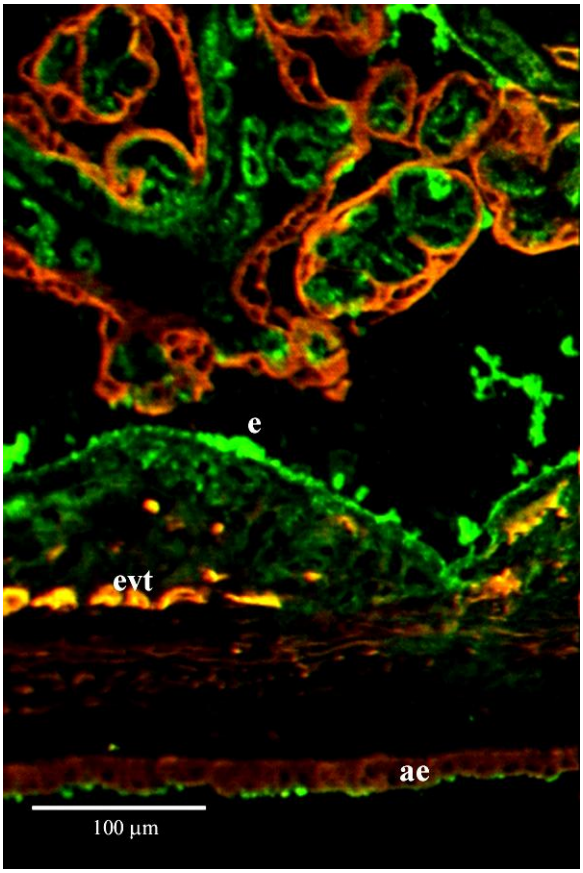

Fig. 3.30 :

Chorionic plate image showing intervillous surface occupied by a structure that appears to be acellular (**e**) but is reactive with anti-von Willebrand factor antibody. This is probably fibrin deposit with blood clotting factors including factor VIII trapped in it. (**ae** = amniotic epithelium; **evt** = extravillous trophoblast).

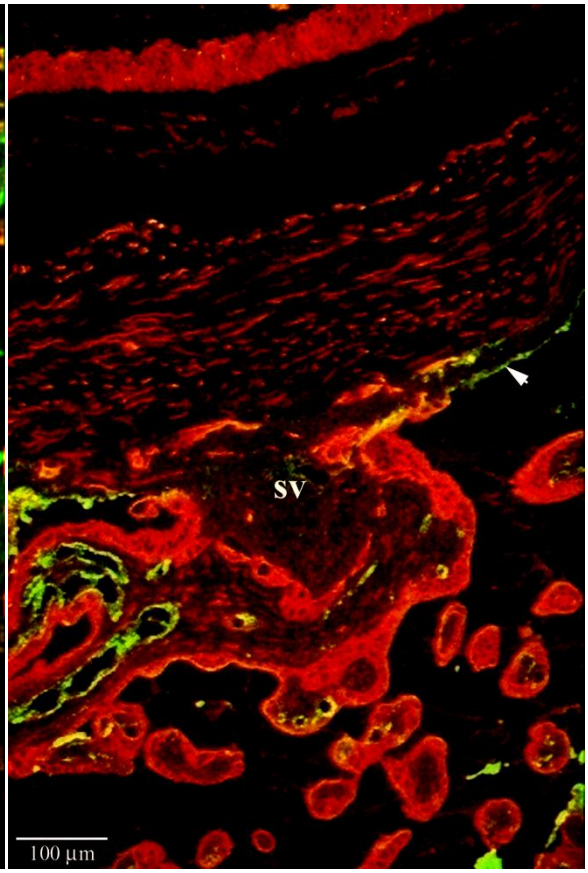

Fig. 3.31:

This is image of chorionic plate showing anti-pancytokeratin (**red**) outlining a stem villous (**sv**) projecting from the intervillous surface into the intervillous space. The far right side of the intervillous surface is lined by anti-von Willebrand factor positive cells (**arrowhead**). The layer in the segment is thin and this is typical of endothelial cells.

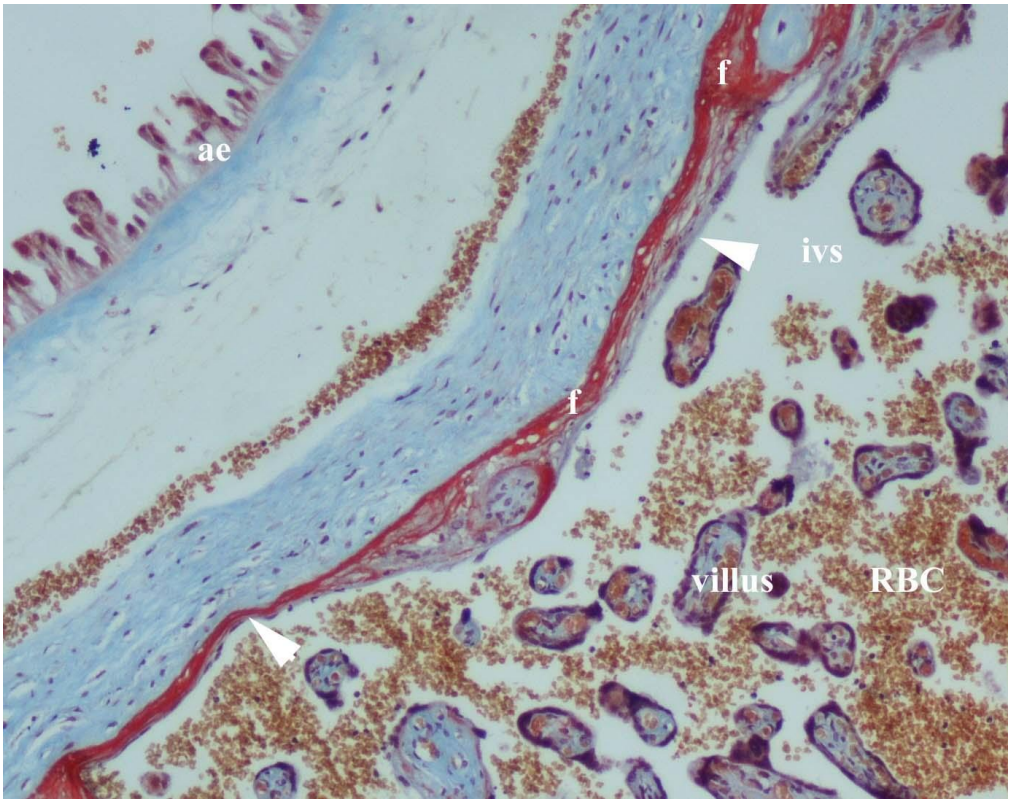

Fig. 3.32: Martius scarlet blus staining of placental CP including part of the IVS showing fibrin (f) deposit long the intervillous space border, just above the cellular lining. Ta some place there appears to be no intervening cellular layer of the IVS

Quantitative Results

Measurement of the chorionic plate intervillous space surface lining components shows that the mean length percent of endothelium, trophoblast and fibrin covering this surface are 27.62%, 36.27% and 36.11 % respectively (Table **3.8** below). Analysis of the data shows that there are no significant differences between the means of the lengths of the three measured segments of the lining of the intervillous surface of the chorionic plate in the placental samples, [f (2, 18) = 1.30, p = 0.298] (Table 3.9).

Table 3.7: Measured components of the chorionic plate intervillous surface

Within-Subjects Factors

Measure: MEASURE_1

Tissue type	Dependent Variable
1	NS
2	Endothelium
3	Trophoblast

These are the names of component tissue types lining the chorionic plate intervillous space. NS represents Fibrin/fibrinoid which are segments negative for endothelial and trophoblast markers

Table 3.8 Descriptive statistics for the chorionic plate IVS surface lining components

Statistics

		Area percent of endothelium (%)	Area percent of trophoblast cells (%)	Area Percent of Non-endothelium and non-trophoblast (%)
N	Valid	7	7	7
	Mean	27.62	36.27	36.11
	Std. Error of Mean	5.82	2.62	4.00
	Median	29.15	33.74	35.20
	Std. Deviation	15.39	6.93	10.58

These are the descriptive statistics for the segment of the chorionic plate intervillous space lining. The mean lengths for the three tissue types are not very different.

Table 3.9: Results of analysis comparing the mean lengths of endothelium, trophoblast and fibrin of the chorionic plate IVS surface lining.

Dependent Variable:Length percent of the CP IVS lining Components

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	342.610[a]	2	171.305	1.295	.298
Intercept	23332.667	1	23332.667	176.358	.000
Liningcomponets	342.610	2	171.305	1.295	.298*
Error	2381.451	18	132.303		
Total	26056.728	21			
Corrected Total	2724.061	20			

a. R Squared = .126 (Adjusted R Squared = .029)

The table shows results of analysis of the data for the CP. There is no statistically significant differences in the mean length percent of the different components lining the chorionic plate intervillous surface as the p values (*) is far greater than 0.05

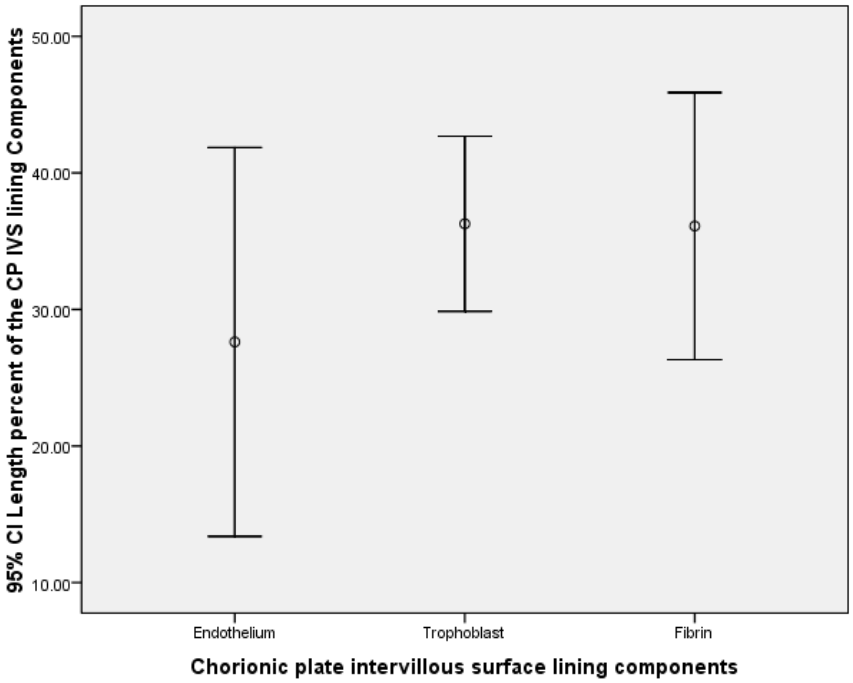

Fig. 3.33: This shows length percent of endothelium, trophoblast and fibrin/fibrinoid lining the intervillous surface of the chorionic plate. The circles show the means and the vertical bars represent 95% confidence interval (CI).

4 DISCUSSION

Specificity of the antibodies

The use of keratin and anti-von Willebrand factor antibodies in this study further establish the use of these antibodies to study trophoblast and endothelium. Although von-Willebrand factor (factor VII) is not specific as it can be found in other sources such as plasma, α-granule of platelets and subendothelial connective tisssues (Sadler, 1998), the fact that it produces a labelling pattern that is comparable to that of EN4 (PECAM 1) which is a more specific endothelial marker and that the labelled cells also have the typical morphology of endothelial cells confirms the reliability of the results.

Composition of the basal plate intervillus surface lining

This study demonstrated that pre-eclampsia is associated with an increase in the mean proportion of fibrin/fibrinoid covering the intervillus surface of the basal plate. On the other hand, there is reduction in the mean length percent of the anchoring villi making contact with the basal plate in pre-eclampsia.

The study results have confirmed the presence of a mosaic unicellular layer comprising of endothelium and trophoblast lining the basal plate intervillous surface (Byrne *et al.*, 1998, Byrne *et al.*, 2001, Smith *et al.*, 2004, Richani *et al.*, 2007). Similarly, the finding of a larger proportion of endothelium (62.8%) than trophoblast (27.6%) in this layer in healthy control and mild pre-eclamptic placentae (69.8% and 20.8%) is consistent with the results of Smith et al (2004) who found the endothelium to occupy 60.8 % of the basal plate and trophoblast forming 18.9% of the lining proportion in control placentae. Richani et al (2007), from another independent laboratory likewise reported the predominance of endothelium (46.5%) over trophoblast (and 27.7%) in control placentae although the proportion of endothelium in their report is slightly lower. The difference between my data and that reported by Richani et al (2007) is probably due to differences in experimental technique. While I used fresh frozen

tissue for immunofluorescence, they used formalin-fixed, dehydrated and wax-embedded tissues and immunohistology. Paraffin embedded tissues are less sensitive to antibodies since wax may mask the antigenic sites in the tissue unlike fresh frozen specimen used in this study and that by Smith et al (2004). Volume changes during dehydration depend on the degree of hydration of different cells and matrices and can cause differential shrinkage and so lead to inaccuracy in comparative measurements. The study by Smith et al(2004)) measured length fractions of fibrin and anchoring villi together and referred to these components under a single entity. It is however sufficient to say that the report of a substantial proportion of the intervillous surface occupied by endothelial cells reported in all these studies is good evidence that neither the all-trophoblast description in most current textbooks (Sadler, 2006, Moore *et al.*, 2008) nor the all-endothelial cell description (Wanner, 1966) reflect reality.

Demonstration of endothelium in samples obtained along the entire radius and from all four quadrants of the placenta suggests that the allo-epi-endothelium covering of the basal plate intervillous surface is a widespread phenomenon and this supports the views of Smith et al.(2004) New evidence in this study has also shown that endothelium is present on the chorionic plate intervillous surface lining, further indicating that endothelial lining is not an incidental finding related to the marginal sinus or the opening of vessels in the basal plate.

Since the placenta is formed from both maternal and foetal tissues it was unclear, initially whether the endothelial cells were of maternal or foetal origin. Unpublished data obtained from a cytogenetic study using y-chromosome probes (Byrne *et al.*, 2006) and the finding by Richani et al (2007) have confirmed that the endothelial cells found at the surface of the basal plate are of maternal origin. What is unclear is how the cells get to this site. There is the suggestion that these might be displaced endothelial progenitor cells from the openings of spiral arteries that open into the intervillous space and maternal veins that drain the intervillous space (Smith *et al.*, 2004). These were thought to be the endothelial cells

replaced by endovascular trophoblast cells during the process of remodelling of the utero-placental vessels. Another view is that these could be trophoblast cells that have transformed into endothelium (vascular trophoblast) (Dye *et al.*, 2001). The argument against the latter view is that these cells are now known to be of maternal origin (Richani *et al.*, 2007) meaning they cannot be transformed trophoblast cells which are of foetal origin. The finding of the endothelial cells distant from the opening of the vessels may suggest that these cells could be, at least partly, from other sources remote from the utero-placental vessels. In this regard, another suggested source is endothelial progenitor cells circulating in the maternal vascular system (Richani *et al.*, 2007). New evidence in this study shows that endothelial cells are a significant component of the chorionic plate intervillous surface lining. As this is the part of the intervillus space lining most remote from the openings of the spiral arterioles in the basal plate these observations and measurements provide further evidence that the endothelial cells may not only be coming from the openings of maternal vessels that supply blood to and have direct access to the intervillous space. Wherever these cells might be coming from, they probably replace syncytiotrophoblast cells that line lacunar systems from which the intervillous space develops.

It is not known if these endothelial cells share the same phenotype as the endothelial cells found in systemic vascular system in terms of their surface molecules, secretory products, and general functions. This could be the subject of future studies. Cells lining parts of the basal plate intervillous surface are known to be reactive with many endothelial markers such UEA-I, thrombomodulin, PAL-E, vWF, P-SELECTIN, E-selectin, ICAM-1, CD44, CD31 and caveolin-I, and they have been described as vascular trophoblast (Dye *et al.*, 2001). It is also unclear whether these cells are lymphatic endothelial cells or blood vascular endothelium. The presence of commercial lymphatic endothelial markers will be useful in differentiating between the two.

It is not known whether these endothelial cells perform a function specific to them at this location. The intervillous space is, as it were, an extension of the maternal vascular space. The endothelial cells found here could be performing equivalent functions of systemic endothelial cells such as selective barriers, control of haemostasis (coagulation and fibrinolysis), regulation of vascular tone, a role in inflammation and new vessel formation (Carine, 2003). As cells that replace trophoblast cells that lined the surface in early pregnancy, I suggest that they are cells involved in repair of damaged trophoblast cells. Hence these endothelial cells could be a source of stem cells that could be used in the treatment of vascular lesions.

Part of the basal plate intervillous surface is covered by fibrinoid. The mean length percent of fibrin/fibrinoid in the control placentae is 5.93% and this is comparable to 5.1% in earlier study (Richani *et al.*, 2007). This fibrinoid is fibrin-type fibrinoid (Frank *et al.*, 1994, Lang *et al.*, 1994) and it is part of the superficial layer of basal plate (Rohr's fibrinoid). It is formed largely from clotting process in the maternal blood in the intervillous space (Frank *et al.*, 1994); therefore the amount deposited is determined by factors regulating intervillous space haemostasis i.e. coagulation and fibrinolytic systems. Generally, intervillous space fibrinoid is deposited from the 12-13 week of gestation (Boyd and Hamilton, 1970) and in normal pregnancy the activation of coagulation increases with gestational age as does the activation of the fibrinolytic system (Mercelina-Roumans *et al.*, 1996) but the balance is tipped in favour of coagulation towards the end of pregnancy (Shaper *et al.*, 1965 , Oliver *et al.*, 1976, Fletcher *et al.*, 1979) and this is a physiological adaptation to cope with haemorrhage associated with separation of the placenta from the wall of the uterus during the third stage of labour. This layer of fibrinoid is probably laid down at places of trophoblastic loss as is the case for perivillous fibrin-type fibrinoid (Nelson *et al.*, 1990, Nelson, 1996, Brownbill *et al.*, 2000, Mayhew *et al.*, 2000). Perivillous fibrinoid deposit was said to initiate re-

epithelialization at sites of epithelial denudation (Nelson *et al.*, 1990, Farmer and Nelson, 1992) by differentiation of cytotrophoblast cells which acts as precursors. On the basal plate intervillous lining there are no cytotrophoblast cells, so damaged trophoblast cells may be replaced by endothelial cells *faux de mieux.*

Placental fibrinoid has been ascribed different functions (Kaufmann *et al.*, 1996a). The superficial fibrinoid layer of the basal plate regulates intervillous blood flow (Stark and Kaufman 1974) and acts as a barrier to extravillous trophoblast migration and immunological barrier that prevent foetal antigen from coming into contact with maternal tissues/antibodies (Benirschke and Kaufman 2000). Perivillous fibrinoid's functions includes maintaining mechanical stability of the placenta (Demir *et al.*, 1997, Benirschke and Kaufman 2000), maintaining feto-maternal barrier (Nelson *et al.*, 1990, Brownbill *et al.*, 1995, Brownbill *et al.*, 2000), structural development and repair of damage to villi (Nelson *et al.*, 1990, Farmer and Nelson, 1992) and regulation of intervillous haemodynamics (Fox, 1967).

The effect of pre-eclampsia on the composition of the basal plate intervillous surface lining

The most significant finding in this study with regards to the effect of pre-eclampsia on the composition of the basal plate intervillous surface lining is reduction in the mean length percent of anchoring villi and increase in the mean length percent of fibrin in the disease. The differences in the means of these two measured parameters between the patients groups are not significant (ANOVA $p = 0.086$ for AV and 0.082 for fibrin) probably due to small sample size. But combining the two by calculating the ratio of AV/NS has helped to overcome lack of power in the individual raw data. In this way, the mean of AV/NS data that was subsequently generated is significantly different between the three groups (ANOVA, $p = 0.010$). Severe pre-eclampsia cases have a lower ratio than the control cases (Posthoc, $p = 0.009$). Furthermore, the mean of NS – AV is statistically different between the groups (ANOVA, $p= 0.033$). This study therefore reveals an increased deposition of fibrinoid on the

basal plate intervillous surface as well as a decrease in the area of anchoring villi that make contact with the basal plate in pre-eclampsia. This finding is supportive of the finding that placental fibrin deposition is increased in hypertensive syndromes in pregnancy (Correa *et al.*, 2008). This is however the first study linking fibrin deposition on the basal plate intervillous surface and area of anchoring villi with pre-eclampsia.

Fibrin deposition in the intervillous space is determined by the balance between blood coagulation and fibrinolytic systems that maintain placental haemostasis. Factors that favour coagulation and/or inhibit fibrinolysis will increase the deposition of fibrin/fibrinoid in the placenta and hence on the intervillous surface of the basal plate and vice versa. Pregnancy in general and pre-eclampsia especially, is characterised by a maternal hypercoagulable state (Redman *et al.*, 1978, Douglas *et al.*, 1982, De Boer *et al.*, 1989). This is because trophoblast secretes tissue factor (TF) (Carson and Ramsey, 1985, Reverdiau *et al.*, 1995), and phosphatidylserine is exposed on the surface of trophoblast during differentiation and both molecules are procoagulant. In addition, pregnancy is associated with a generalised systemic inflammatory response (Redman *et al.*, 1999) and pre-eclampsia is characterised by generalised maternal endothelial cell dysfunction (Roberts, 1998). This procoagulant state is however counterbalanced by local inhibitory mechanisms such as secretion of Thrombomodulin (TM) (a cofactor for activation of protein C by thrombin) (Salem *et al.*, 1984, Maruyama *et al.*, 1985, Fazel *et al.*, 1998, Lakasing *et al.*, 1999), Anexin V (Krikun *et al.*, 1994, Rand *et al.*, 1994, Rand *et al.*, 1997, Lakasing *et al.*, 1999) and Tissue factor pathway inhibitor type 1 and 2 (TFPI-1 and TFPI-2) (Drake *et al.*, 1989, Udagawa *et al.*, 1998, Edstrom *et al.*, 2000). Fibrin deposited as result of coagulation is broken down by fibrinolytic protein plasmin, which is formed from activation of the proenzyme plasminogen by tissue-type (tPA) and urokinase-type (uPA) plasminogen activators, into fibrin degradation products (FDP). The placenta is a known source of endothelial cell-specific tPA

and placental-specific uPA (Jonasson *et al.*, 1989, Hofmann *et al.*, 1994, Hu *et al.*, 1999) and also their inhibitors, Type 1 (PAI-1) and Type 2 (PAI-2) (Astedt *et al.*, 1985, Astedt *et al.*, 1986, Estelles *et al.*, 1994, Estelles *et al.*, 1998, Hu *et al.*, 1999).

In pre-eclampsia it is the fibrinolytic system which is deranged (Lanir *et al.*, 2003). Pre-eclampsia is generally associated with increased activity of procoagulant and antifibrinolytic systems resulting in promotion of fibrin deposition (Estelles *et al.*, 1989, Belin, 1993, Estelles *et al.*, 1994, Kanfer *et al.*, 1996, Estelles *et al.*, 1998). It has been shown that in severe pre-eclampsia, plasma concentration and the activity levels of PAI-1 is significantly increased compared to normal pregnancy (Estelles *et al.*, 1989). Also the antigen levels of PAI-1, tissue factor (promoter of coagulation), tumour necrosis factor alpha (TNFα) and the mRNA of PAI-1 and tissue factor (TF) in placentae from pre-eclamptic patients are elevated (Estelles *et al.*, 1998). Furthermore, placental plasminogen activator production is significantly decreased in pre-eclampsia (Yoshimura *et al.*, 1985). All this supports the increased deposition of fibrin in pre-eclampsia.

Pre-eclampsia and all pregnancy states are associated with a fall in fibrinolytic activity and a rise in fibrinogen levels in the blood but in women who develop pre-eclampsia there is delay in the return of these factors to normal levels after delivery (Condie and Ogston, 1976). There is also impaired release of plasminogen activator from the endothelial cells of women who develop pre-eclampsia during pregnancy (Condie, 1976). These two features of pre-eclampsia favour coagulation and deposition of fibrin in the placenta.

In addition, pre-eclamptic placentae produce about seven times more thromboxane than prostacyclin while normal pregnancy placenta produces equivalent amounts of thromboxane and prostacyclin (Walsh, 1985). Since prostacyclin antagonises platelet aggregation by thromboxane, there is therefore increased platelet aggregation and clot formation in pre-eclampsia compared to normal pregnancy.

Increased placental fibrin deposition, as seen in this report, could be due to other features of pre-eclampsia. For example pre-eclampsia is associated with poor perfusion of the intervillous space as a result of incomplete physiological transformation of the spiral arterioles that supply blood into the intervillous space (Brosens *et al.*, 1972, Robertson *et al.*, 1985, Khong *et al.*, 1986, Meekins *et al.*, 1994, Matijevic and Johnston, 1999). This will eventually result in decreased intervillous space blood flow leading to stasis and hence increased coagulability of the blood and increased deposition of fibrin in the intervillous space. Secondly, Rohr's fibrinoid underlies the cellular lining of the basal plate intervillous space lining layer (Boyd and Hamilton, 1970). Damage to the cells in this layer without compensatory replacement by the same or another cell type will expose the fibrinoid layer to the intervillous space. In this way, there will be gaps within the cellular lining filled with fibrinoid. Pre-eclampsia is associated with hypoxia and oxidative stress with resultant damage to trophoblast cells including damage to the cellular layer of the basal plate intervillous lining cells (Hung *et al.*, 2001, Hung *et al.*, 2002, Burton and Hung, 2003, Jauniaux *et al.*, 2006). The length percent of the lining that is covered by fibrin may be predicted to increase in pre-eclampsia.

The current finding of increased deposition of fibrin on the basal plate of placentae from pre-eclamptic pregnancies is consistent with a state of reduced fibrin clearance and increased coagulation in the disease.

The clinical significance of the increased fibrinoid deposition over the intervillous border of the basal plate in pre-eclamptic placentae seen in this study is not clear. The finding in this report that there is an associated reduction of the length proportion of anchoring villi with the increase length percent of fibrin in pre-eclampsia compared to healthy controls is evidence that fibrin could play a significant role in development of pre-eclampsia. This layer of fibrinoid (Rohr's fibrinoid) is never invaded by trophoblast cells (Frank *et al.*, 1994) and

therefore thought to function as a barrier to extravillous cytotrophoblast invasion and immunological barrier to foetal paternally-derived antigens (Kaufmann *et al.*, 1996a), a similar role proposed for intra-tumour fibrin (Dvorak *et al.*, 1983). Increased deposition of fibrin/fibrinoid within the basal plate, particularly on the intervillous surface will lead to an increased surface area of the fibrin barrier and therefore impairment of anchoring villi development and subsequent invasion and remodelling of spiral arteries by extravillous trophoblast cells, which gain contact with the basal plate through anchoring villi. This will lead to poor placentation, a phenomenon which is fundamental to systemic pre-eclampsia development (Burton and Jauniaux, 2004, Redman and Sargent, 2005, Jauniaux *et al.*, 2006).

To be able to impair placental formation and hence cause pre-eclampsia, massive amounts of fibrin in the placenta must form early in gestation at the time of placental formation. At the moment there are apparently no studies that have compared fibrin deposition in the first trimester placenta in women who later develop pre-eclampsia with those who did not. Increase in the fibrin component in the pre-eclamptic group will confirm the etiological role of fibrin. Unfortunately this cannot be done now as there are no non-invasive ways to measure the length of fibrin here. Until that is developed the essential role of fibrin will remain unproven. The finding of increased deposition of fibrin in severe pre-eclampsia compared to control placentae is however, evidence against the view that placental fibrin deposition generally, is independent of pregnancy outcomes (Kaufmann *et al.*, 1996a). It is known that massive perivillous fibrin-type fibrinoid deposition occurs in placenta of diabetic women, a condition with poor foetal outcome (Mayhew and Sampson, 2003). Massive perivillous fibrin deposit is a common pathological association with miscarriage and intra uterine foetal growth retardation (IUGR) due to failed anticoagulant treatment of antiphospholipid antibody syndrome (Salafia, 1997).

If the etiological role of placental fibrin is confirmed, it will justify and explain the use of anti-platelet drugs such as aspirin in high risk patients to prevent pre-eclampsia and in diagnosed cases to limit severity of the disease. Currently there are reports of favourable outcomes for the use of aspirin in prevention and limiting the severity of pre-eclampsia especially when started in the first trimester in high risk groups (Vainio *et al.*, 2002, Chiaffarino *et al.*, 2004, Ebrashy *et al.*, 2005, Askie *et al.*, 2007).

It has also been suggested that fibrin deposition in tumours acts as an immunological barrier by inhibiting tumour antigens from coming into contact with host antibodies (Dvorak *et al.*, 1983). Immunological mechanisms in the pathophysiology of pre-eclampsia are well described (Trupin *et al.*, 1996, King *et al.*, 1998, Söderström-Anttila *et al.*, 1998, Hiby *et al.*, 1999, King *et al.*, 2000a, King *et al.*, 2000b, Moffett-King, 2002, Hiby *et al.*, 2004). Increased fibrin deposition in the disease could well be a reaction to immunological derangement in the disease.

The evidence from this study suggests that pre-eclampsia has no significant effect on the length percent of endothelium and trophoblast cells lining the basal plate intervillous space surface (mean length of 69.8% for endothelium and 20.8% for trophoblast in mild pre-eclamptic and 62.8% of endothelium and 27.6% of trophoblast in controls). This is not consistent with a report of exaggeration of the length proportion of endothelium in pre-eclampsia (Smith *et al.*, 2004) and the tendency for higher proportion of trophoblast and a reduction of endothelium/trophoblast ratio (Richani *et al.*, 2007) associated with the disease reported in earlier studies. The difference in these findings could be explained by differences in the choice of patients, sample size, sample preparation and method of measurement of the images. Severity of pre-eclampsia also has no significant effect on the length percent of the cellular components of the lining of the basal plate intervillous surface. This is the first study comparing the length percent of the cells lining the intervillous surface of the basal plate in

mild and severe pre-eclampsia. Although there is a tendency of reduction in the length percent of endothelial cells in the severe patients group (51.2%) compared to mild pre-eclampsia (69.8%) and control (62.8%) statistical inferential tests revealed there is no significant difference probably due to small sample size. A study involving larger sample size will therefore be required to fully assess the effects of pre-eclampsia in general and severe pre-eclampsia on the composition of the basal plate intervillous surface lining cells. Before that is done my view on the effect of pre-eclampsia on the composition of the cells lining the basal plate intervillous surface is that this will vary from patient to patient depending on which feature of the physiology of pregnancy is deranged. Pre-eclampsia will lead to increase in the proportion of endothelial cells when the placental circulation is not extremely compromised so that utero-placental perfusion is adequate for endothelial cells from the ends of the uteroplacental vessels and endothelial progenitor's cells in the circulation will enter the intervillous space to replace damaged trophoblast cells. In cases in which the placental circulation is severely compromised with reduced intervillous space perfusion and placental oxidative stress, large segment of the syncytiotrophoblast cells lining the intervillous surface of the basal plate will be damaged and will not be replaced adequately by endothelial cells and the fibrin component will expand. The fact that, the length percent of fibrin deposited on the BP correlates possitvely with the the level of maternal SBP points to this fact (refer to table 3.5 and fig. 3.22).

The finding here that in the severe pre-eclampsia group there is no significant difference between the length proportion of endothelium and trophoblast is interesting and may mean that in this class of patients endothelial cell population of the lining is impaired. Length of endothelial cells lining the BP IVS surface appears to show negative correlation with the level of maternal SBP. Richani et al (2007)has found that there is reduction in the length proportion of endothelium and increase in the trophoblast proportion in pre-eclampsia. The

result of this study however shows only a trend towards reduction in the proportion of endothelium (51.2%) and increase in the proportion of trophoblast (35.4%) in the severe pre-eclamptic placentae when compared to that of control but these differences are however not statistically significant.

Chorionic plate intervillous surface lining.

The specific labelling of the two characteristic components, trophoblast and endothelium, of the lining of the chorionic plate intervillous surface have established for the first time that chorionic plate intervillous surface lining is composed of a unicellular layer comprising of endothelium, trophoblast and an acellular fibrinoid material that is negative for both endothelial and trophoblast markers. The lining of the chorionic plate intervillous surface described here is similar to the mosaic described for the human placental basal plate (Smith *et al.*, 2004). Clearly on the basis of this data the mosaic extends beyond the basal plate.

This layer was described as made of syncytiotrophoblast cells and fibrin (Bourne, 1962, Bourne, 1966, Boyd and Hamilton, 1970, Benirschke and Kaufman 2000). The finding of a fairly large area of the lining covered by endothelial cells (27.62%) (**Table 3.8**) is important and shows that the current description of this layer in textbooks as comprising of trophoblast and fibrin could not be substantiated.

The data shows that the mean length of endothelium lining the chorionic plate intervillous surface is 27.62 % compared to 62.83 % over the basal plate intervillous surface (**Tables 3.5 and Table 3.8**). It is therefore lower compared to the basal plate intervillous surface lining endothelium found in this study as well as previous reports (Smith *et al.*, 2004, Richani *et al.*, 2007). This proportion is a however significant and together with the fact that endothelium is present in samples taken from all over the entire chorionic plate surface is strong evidence against previous description of trophoblast and fibrin (Bourne, 1962, Bourne, 1966, Boyd and Hamilton, 1970, Benirschke and Kaufman 2000).

The origin of the endothelial cells found here is not known, but as a guess, they are of maternal origin as is the case for the basal plate (Byrne *et al.*, 2006, Richani *et al.*, 2007). They probably originated from the ends of the vessels that open into and drain the intervillous space (Smith *et al.*, 2004, Richani *et al.*, 2007). They could also be coming from the endothelial progenitor cells in the maternal circulation (Richani *et al.*, 2007). Wherever these cells might be coming from, they appear ultimately to replace the syncytiotrophoblast cells that line this layer in the developing placenta.

The cotyledonary placenta is separated into lobes by septae of maternal origin that resisted the erosive progress of implantation and placentation. To complete a functional lining to the intervillous space, the lining layer must cover not only the basal and chorionic plate and the villous tree but also these septae. These septal bulkheads are orthogonal to the chorionic and basal plates. In order to enclose the maternal blood the lining layer must be continuous and waterproof but not necessarily entirely cellular, as fibrin will form waterproof clots where damage to the cellular lining exposes underlying extracellular matrix.

Conclusions

This study used appropriate sampling method to confirm that the intervillous surface lining mosaic monolayer (endothelium and trophoblast) is present throughout the basal plate and for the first time, described the presence of endothelium lining a significant proportion of the chorionic plate intervillous surface. Fibrin area fraction of the basal plate intervillous surface lining showed tendency to increase in pre-eclamptic, especially in severe pre-eclampsia placentas, compared to healthy control placentas. Anchoring villi area fraction of the basal plate intervillous surface on the other hand showed tendency to decrease in pre-eclamptic placentas compared to healthy controls. Independently however, the means of these two basal plate components are are not significantly different between the groups. The means of the ratio of length percent anchoring villi to length percent of fibrin (AV: NS) was however

significantly different between the groups. The mean of AV: NS ratio decreases significantly with increasing severity of pre-eclampsia. This inverse relationship between BP fibrin deposition and anchoring villi attachment area indicates that dysregulation of fibrin deposition and poor trophoblast invasion are involved in the disease progression. It has also been confirmed here that pregnancies complicated by pre-eclampsia are associated with low birth weight babies and iatrogenic prematurity as the pregnancies are terminated before term as means of treatment.

Limitations

The stringent criteria set for recruiting only pure primigravidae pre-eclamptic patients into the study group make it difficult to recruit adequate numbers. In addition to this, severe pre-eclamptic patients are usually admitted and managed on an emergency basis and are therefore delivered at unpredictable times shortly after admission to hospital, and possibly at unsocial hours. Because they are emergencies, it is difficult for the labour ward staff to liaise with researchers when a placenta is delivered and the placenta may be prepared in a manner optimised for the neonatal pathologist and unsuitable for random sampling research methods. This makes it difficult to recruit adequate numbers of pre-eclamptic patients in a single centre and most especially severe pre-eclamptics for the study. The analysis of the data shows that the length percent of endothelium in the severe pre-eclampsia (51.2%) is not statistically different from that of mild pre-eclampsia (69.8%) probably owing to small sample size. Further recruitment could not be carried out during the study period in order to boost the statistical power of the study design.

Gestational age was thought to affect the composition of the basal plate intervillous surface lining. Most of the severe pre-eclamptic patients were delivered preterm unlike the controls and the mild pre-eclamptics which were delivered at term. Hence the severe group could not be gestationally matched with the control and the mild pre-eclamptics. However, the

previous study by Smith et al (2004) did not find any correlation between gestational age and the length percent of endothelium and trophoblast cells.

Dual-labelling employed in this study makes it difficult to accurately determine the real length of fibrin forming part of the lining surface. Areas of cellular lining that are damaged through tissue preparation could appear as unlabelled and then included in the fibrin data. Similarly if a segment of the cellular lining is out of the tissue plane and therefore out of focus, it will appear void and unlabelled and again be included in the fibrin data. This could be avoided if three channels labelling with three different antibodies and fluorophores are applied simultaneously. The fact that the length percent of fibrin in this study, (5.93%) is comparable to 5.1% obtained by Richani et al (2007)using a different antibody, however makes the data obtained likely to be quite accurate and reproducible.

Future Work

It will be necessary to recruit larger numbers of both pre-eclamptics and control placentae in order to fully evaluate the effect of pre-eclampsia and severity of pre-eclampsia on the composition of the basal plate intervillous surface lining.

A study to find out whether the endothelial cells found in the placenta and specifically on the intervillous surface lining are of vascular or lymphatic origin by applying lymphatic endothelial markers. An added benefit will be finding whether the placenta has lymphatic drainage at all.

Another thing that needs clarifying and which can be resolved by future work will be to make similar measurements on first and second trimester placental specimens and compare the length percent of the components of the lining with term or near term specimens.

Another interesting thing that will engage future attention is to describe at the ultrastructural level the nature of the junction between endothelial cells and trophoblast cells at the basal plate intervillous surface lining.

Lastly I would like find out the origin of the endothelial cells lining the chorionic plate intervillous surface lining.

5 APPENDICES

APPENDIX 1: PATIENTS RECRUITMENT

The following patient information leaflet was give to every patient that was recruited into the study. Patients were then asked to sign a consent form administered by me. The two forms are shown below.

PATIENT INFORMATION LEAFLET

PATIENT INFORMATION LEAFLET, VERSION NO 2003CDO2, Thursday, 18th November 2004
Study No LGH 9161 LLREC 7144

Study title: Pre-eclampsia: a disease that causes high blood pressure and protein in the urine of pregnant women and may lead to harm for their babies

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Principal Investigators *Dr Colin Ockleford PhD, FRCPath*

Mr Paul Bosio MD MRCOG

Mr Jason Waugh MRCOG

For further information you may contact Dr Ockleford at:-

The Advanced Light Microscope Facility

Maurice Shock Medical Sciences Building

Leicester Warwick Medical School

University Road

Leicester LE1 9HN

Tel: 0116 252 3020

Email: cxo@le.ac.uk

You are being invited to take part in a research study that will only require the donation of a few sugar-cube sized tissue samples of the after-birth (to be removed immediately prior to is disposal). Before you decide to participate 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 and discuss it with others if you wish. 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.

1. What is pre-eclampsia?

Pre-eclampsia is a disease that can affect pregnant women. In mild cases it causes protein in the urine, swelling of tissues and slightly raised blood pressure. Untreated, the raised blood pressure can have severe effects. In these cases it can lead to eclampsia where the mother has fits and harm can come to her baby. Although the cause is not yet fully understood it is believed to involve afterbirth tissue as where that exhibits certain particular alterations pre- eclampsia is more common. Pre- eclampsia is obviously a disease where progress in understanding, if it leads to new treatments, could be very valuable.

2. What is the purpose of the study?

We have recently uncovered new information about the detailed structure of the tissues in the healthy human after-birth. The new information relates to a part of the placenta that develops abnormally when pre-eclampsia ensues. We now wish to study this area in detail comparing the normal and pre-eclamptic afterbirth very carefully to see what we can learn

about the course of the disease. At some future stage this may help us to develop new rational strategies for treatment of the disease. The study (Leicester Research Ethics Committee ref. No. 6336) of which this is a development was originally sponsored by "Birth Defect foundation" The work is to be carried out by staff at Leicester Warwick Medical Schools.

3. Why have I been chosen?

You have been chosen either

a) because you have clinical signs of pre-eclampsia and we would like to examine small samples of afterbirth after it is delivered for changes associated with the disease. Or alternatively
b) because your pregnancy is a healthy one and we wish to compare similar small samples of afterbirth taken after it is delivered so that we can be sure we have a good basis for comparison.

4. What will happen to me if I take part in the study?

- The whole study should be complete within 3 years of the start date in September 2007.
- The number of visits to hospital will not be increased beyond that expected for your normal treatment.
- Any tests to be carried out, e.g. physical examination, blood test etc. on you and your baby will be the normal routine ones for your own and your baby's benefit. In addition we would like you to give permission for use of samples of the afterbirth. These will be obtained after the delivery of the afterbirth and baby for careful microscopic and cell-biological study. Subsequently the tissue of the afterbirth will be disposed of in the approved manner for human tissues. We do not plan to keep any tissue beyond the end of the study this will be at the latest 3 years from your giving consent.
- You will not be asked to keep any records specifically for this study.
- You will not be asked to fill in any questionnaires for the purposes of this study.
- The procedures for our research will all be carried out on the afterbirth after your baby has been delivered and special research staff will be handling the tissue. We do not therefore envisage any additional risk involved for you or your baby in helping with this research.
- No short-term benefits will accrue to you or your family. We hope and are working hard to try to ensure that there will be long term ones for others and babies affected by this condition in the future.

4. Will information obtained in the study be confidential?

As usual your treatment will be recorded in your medical records and these will be treated with the usual degree of confidentiality under the data protection act. Some relevant excerpts of your notes may be useful as raw research data to members of the research team. The consultant-in-charge of your welfare and that of your baby will arrange to transmit

only any necessary information and then using a code number. Y*our name and address will be removed so that you cannot be recognised from it.'*

You will not be identified in any documents or published work relating to the research.

Normally the patient's GP is informed of the patient participation in a medical study. As this study is of the afterbirth only we do not intend to do so on this occasion. However should you wish to consult your GP please do so and show them the information you have been given. We will make ourselves available to discuss any issues with your GP if they think it valuable.

5. What if I and/ or my baby are harmed by the study?

We believe this to be extremely *unlikely* as we are only intending to study the afterbirth following your baby's birth. Nevertheless the research will be indemnified in the usual way. If you are harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms are available to you.'

6. Will I receive out of pocket expenses for taking part in the study?

There will be no out of pocket expenses payment associated with this trial and there should be no additional travel involved. Neither will your doctor be paid for including you in this study; he is doing this in the hope that the work will benefit future patients.

7. Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

8. What will happen to the results of the research study?

We aim to publish the outcome of our work in peer-reviewed medical and scientific journals that are freely accessible so that they are most useful to the widest range of people. We cannot predict where they will appear, as acceptance for publication is an editorial decision, but this should be within 4 years and if any member of the team is contacted they undertake to indicate to participants where relevant results are to be, or have been published, so they can obtain a copy.

9. Who has reviewed the study?

All research that involves NHS patients or staff, information from NHS medical records or uses NHS patients or staff, information from NHS medical records or uses NHS premises or facilities must be approved by a Leicestershire Local Research Ethics Committee before it goes ahead. Approval does not guarantee that you will not come to any harm if you take part. However, approval means that the Committee is satisfied that your rights will be respected, that any risks have been reduced to a minimum and balanced against possible benefits and that you have been given sufficient information on which to make an informed decision to take part or not.

'Thank you for reading this.'

Patient name, address, DOB (or ID label)

Centre Number: Research and Development Department, LGH UHL 9161

Study Number: Study No LGH 9161

Patient Identification Number for this trial: _____

CONSENT FORM

Title of Project:

"The Basal Plate and the pre- eclamptic materno- fetal interaction."

Short title: High blood pressure in pregnancy

Name of Researcher / Principal Investigator:

Dr Colin Ockleford, Mr Paul Bosio, Jason Waugh and their research teams.

Please initial box

1. I confirm that I have read and understand the information sheet dated Thursday 18th November, 2004. version NO 2003CDO2 for the above study and have had the opportunity to ask questions. ☐

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 I may withdraw my consent to my tissue being used at any time without justifying my decision and without affecting my normal care and medical management. ☐

4. I agree to donate the tissue samples as detailed below and allow their use in medical research as described in the Patient Information Leaflet. ☐

5. *I understand that the tissue is a gift and that I will not benefit from any intellectual property that results from the use of the tissue.* ☐

6. I understand that tissue samples will not be used to undertake any genetic tests whose results may have adverse consequences on my or my families insurance or employment. ☐

7. **I agree / do not agree** to my tissue samples being used to undertake genetic research as described in the patient information leaflet ☐
(patient to delete as applicable)

8. I understand that tissue samples and associated clinical data may be transferred to non-commercial research partners of the University Hospitals of Leicester NHS Trust and Leicester University Medical School, but that the information will be anonymised prior to transfer. ☐

9. *The samples which I hereby consent to donate are:* ☐

Small samples of placental tissue taken after delivery from the afterbirth. There will be approximately 5 of these small pieces 1cm by 1cm by 1cm (each the size of a sugar cube).

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

_____	_____	_____
Name of Patient	Date	Signature

_____	_____	_____
Name of Parent/guardian/ legal representative ***(If applicable)***	DateSignature	

_____	_____	_____

Researcher	Date	Signature

Original for researcher/site file/CRF

copy for patient, copy for hospital notes

APPENDIX 2: ANTIBODIES AND SOLUTIONS

Primary Antibodies:

Trophoblast markers

Mouse monoclonal anti-pancytokeratin-IgG1 specific (Sigma-Aldrich, Missouri, USA, C2931) at dilution of 1:800

Rabbit (sc-17031-R) polyclonal anti-keratin 18 (p-Cytokeratin 18 Ser 33) (Santa Cruz Biotechnology, Inc.) at dilusion of 1:50.

Cytokeratin

Cytokeratins or Keratins (Ks) are a characteristic cytoskeletal marker of extra-embryonic membrane epithelia and is part of the 10nm diameter bundles of intermediate filaments (Ockleford *et al.*, 1981, Ockleford *et al.*, 1984). They form the cytoskeleton of epithelial cells. They are classified into two types: The basic type II keratins (K1-K8) and the acidic type I keratins (K 9 to K20) (Moll *et al.*, 1982). Most keratins genes occur in pairs and are regulated in tissue and differentiation-specific manner and have been used as a tool in the study of epithelial tissues in health and disease (O'Guin *et al.*, 1990). About 54 keratins are known in humans (28 type I and 26 type II). The keratin genes are located on chromosomes 17 and 12 (Hesse *et al.*, 2001, Hesse *et al.*, 2004, Rogers *et al.*, 2004, Rogers *et al.*, 2005).

The human placental trophoblast is reported to express keratins in developmental, differentiative and functional specific patterns (Muhlhauser *et al.*, 1995). K7 and K18 are known to be consistently present in trophoblast cells. I chose monoclonal pancytokeratin which is immunoreactive to a cocktail of keratins; 4, 5, 6, 8, 10, 13 and 18 in most human epithelial tissues because of the quality of its labelling based on previous studies in the laboratory (2004). Secondly, the antibody was already present in the lab and using the available antibody helped to save cost. Polyclonal rabbit anti-keratin 18 on the other hand

recognises K18 and it was used as a control to compare the labelling pattern of the anti-pancytokeratin antibody.

Other marker proteins for trophoblast cells are human chorionic gonadotrophine (hCG) and placental lactogen (PL).

Endothelial markers

Endothelial cells are flat, pavement-like cells that line the luminal surface of blood vessels and lymphatics. They perform various functions including maintaining a semipermeable barrier between the blood and the rest of the vessel wall and regulation of intravascular coagulation and fibrinolysis. Endothelial cells are heterogenous in gene expression, structure, and their function is specific to the tissue in which they are found (Augustin *et al.*, 1994, Risau, 1995, Rajotte *et al.*, 1998, Chi *et al.*, 2003). Many markers of endothelial cells have been proposed and these includes von Willebrand factor, thrombomodulin (CD141), CD34, VE-cadherin, vimentin and CD31 (PECAM 1), the sulphated epitope MECA-79, the Duffy antigen receptor for chemokines (DARC), CD105 (endoglin), angiotensin converting enzyme (ACE) and CD146.

Anti-human von Willebrand factor was used in this project because it was readily available in the laboratory as it was used in previous studies in the past. Secondly the choice of anti-vWf was because of its suitability for dual-labelled experiment as was demonstrated by Smith et al(2004). vWf is a270kD plasma glycoprotein that mediates platelets adhesion to vascular endothelium and serves as a carrier and stabilizer of cogulation factor VIII (ant-haemophiliac factor). It is secreted by megakaryocytes and it is present in plasma, platelets, endothelium and subendothelial connective tissues (Sadler, 1998).

EN4: A mouse monoclonal $IgG_{1.}$ It is antibody is raised in mouse against human umbilical cord vascular endothelial cells. It recognises platelet/endothelial cell adhesion molecule-1

(PECAM-1) also referred to as CD31. PECAM-1 is a glycoprotein expressed on the cell surface of monocytes, neutrophils, platelets and a subpopulation of T cells (Santa Cruz data sheet).

Secondary antibodies

Cy3-conjugated, sheep anti-mouse IgG. The fluorophore absorbs light in the green wavelength and emits red signals. It binds the Fab_2 fragment of mouse IgG.

Fluorescein thiocyanate (FITC-)- conjugated goat anti-rabbit IgG. It is Fab_2 specific antibody against rabbit IgG.

SOLUTIONS

Tris HCL

Tris (Tris-hydoxymethylmethylamine) is an organic buffer used to maintain pH of antibodies. To prepare Tris HCl dissolve 121.14mg (molecular weight of Tris) of Tris in approximately 800ml of distilled water. To the stock, a solution of HCl is titrated until pH is 7.6. Top the solution up to a litre.

10 molar Tris Buffered Saline (10 ×M TBS)

Add 87.66 g NaCL (ie$10 \times 150mM\ NaCL = 10 \times \frac{58.44g}{1000} \times 150$) to 200 ml of Tris HCL.

Top up the resultant solution with distilled water to 1 litre.

1 molar TBS-T

To 100 ml of the 10 molar TBS, add 900 ml of distilled. To this add 2 ml of Tween 20.

1:1 Acetone: Methanol

Add equal volume of acetone and methanol

APPENDIX 3: DATA

The following clinical data for each recruited patient was obtained from the clinical case notes.

Patient Data

Patients Project Number

Age:

DOB:

Hosp No:

Project No:

EDD:

Parity:

Gestational age at Delivery:

Race

PMH:

Family History:

Smoking history

Gestational age at onset of PE

Current medication:

Highest BP:

Highest Urine Protein:

LFT Result:

Normal

Abnormal:

Coagulopathy

IUGR:

Mode of delivery:

Sex OF Baby:

Outcome of Delivery:

Birth weight:

Apgar Score:

1min:

5min:

Uric Acid:

Table 5.1 Clinical and demographic data for the healthy control patients

Patient	Age	FHPE	HSBP/mmHg	HDBPmmHg	Protein	ONSETPE	GAD/weeks	Sex of baby	Birth weight/g
PC1	19	no	131	81	2+	n/a	40	female	2620
PC2	30	NO	136	76	1+	n/a	40	male	3600
PC3	24	NO	111	74	0	n/a	41	female	3220
PC7	35	no	142	90	2+	n/a	41	female	4138
PC8	21	yes	130	68	0	n/a	31	male	1980
PC9	19	no	120	65	0	n/a	39	female	2849
PC10	22	yes	130	80	0	n/a	37	male	2721
PC11	28	no	120	78	0	n/a	40	male	3480
PC12	24	no	112	68	0	n/a	39	male	3218
PC13	30	yes	126	76	0	n/a	40	male	3162
PC002	20	no	115	82	0	n/a	38	female	2640

FHPE=family history of Pre-eclampsia
HSBP=highest systolic blood pressure
HDBP=highest diastolic blood pressure
Protein= Urine protein-Random or 24hour in g/l
ONSETPE=Gestation age at onset on PE
GAD =Gestational age at delivery

Table 5.2: Clinical and demographic data for the mild pre-eclamptic patients

Patient	Age/years	FHPE	HSBP/mmHg	HDBP/mmHg	Protein	ONSETPE	GAD/week	Sex of baby	Birth weight/g
PE4	27	no	145	101	2+		39	female	3146
PE7	29	no	149	100	3+	37+0	38	male	3090
PE8	17	no	146	105	3+		39	male	3400
PE10	28	no	151	109	3+		40	male	3033
PE15	31	no	155	104	3+		37	female	2720
PE17	30	no	151	93	3+		38	female	2976
PE19	23	no	157	100	1.8g/l	34+5	37	female	2636
PE23	26	no	154	96	1.76g/l-3+	38+3	39	female	4110
PE002	29	yes	147	101	0.91		40	male	3920
PE25	30	no	151	108	1+ or 0.63g/l	38+1	38	female	2976

FHPE=family History of Pre-eclampsia
HSBP=highest systolic blood pressure
HDBP=highest diastolic blood pressure
Protein= Urine protein-Random or 24hour in g/l
ONSETPE=Gestation age at onset on PE
GAD =Gestational age at delivery

Table 5.3: Clinical and demographic data for the severe pre-eclamptic patients

Patient	Age/years	FHPE	HSBP	HDBP	Protein	ONSETPE	GAD/weeks	Sex of baby	Birth weight/g	IUGR
PE2	17	no	170	111	3+		35	male	1880	
PE6	23	no	166	100	3+		39	female	3628	
PE9	17	no	155	109	3.19/3+	32	32	female	1480	
PE11	20	no	154	110	9.73g/3+		36	female	2069	
PE14	30	no	155	106	0.61g	33+0	37	male	2409	PE14
PE20	22	no	163	118	5g/l	36+2	38	male	2863	yes
PE21	27	no	180	115	11.97/3+	30	31	female	1020	
PE22	30	no	172	106	0.76g/24hrs	34+6	35	female	1820	yes
PE28	23	no	156	112	3.07g/24hrs	32	**33**	male	1080	
PE27	23	no	162	108	3+		35	female	1878	
PE26	20	yes	172	112	4.12g/3+		32	female	1920	

FHPE=family History of Pre-eclampsia
HSBP=highest systolic blood pressure
HDBP=highest diastolic blood pressure
Protein= Urine protein-Random or 24hour in g/l
ONSETPE=Gestation age at onset on PE
GAD =Gestational age at delivery

Table 5.4: An example of the raw data for control patient: PC2B

	END	TPB(µm)	AV(µm)	NS(µm)
	371.78	88.15	98.91	45.09
	61.16	48.8	39.52	46.99
	85.75	43.08	23.18	25.91
	44.12	240.57	31.71	87.32
	23.44	296.47		66.05
	327.33	118.55		17.97
	218.51	318.3		30.72
	336.38	86.66		161.68
	41.96	89.16		97.59
	43.82	49.04		119.72
	187.69	350.45		58.74
	214.55	93.22		230.52
	79.61	128.32		312.06
	340.34	126.2		30.12
	82.7	46.51		23.02
	55.54	90.38		41.61
	161.41	93.77		89.52
	96.65	219.06		17.91
	338.89	40.05		
	44.6	70.52		
	217.19	53.95		
	86.97	115.6		
	330.25			
	398.74			
	184.19			
	154.56			
	113.29			
Total	**4641.42**	**2806.81**	**193.32**	**1502.54**
Length percent	50.76	30.7	2.11	16.43

Table 5.5: An example of the raw data for mild pre-eclamptic patient: PE25

	END(µm)	TPB (µm)	AV(µm)	NS(µm)
	402.42	200.15	100.9	311.46
	356.92	508.16		294.46
	422.39	654.14		116.4
	591.68	428.16		24.1
	656.18	295.71		268.7
	658.03	96.17		252.22
	580.47	240.6		198.32
	73.41	102.08		245.75
	696.64	443.47		126.89
	217.39	440.99		175.31
	117.48	205.05		
	677.34	956.44		
	318.87	109.65		
	300.89			
	437.3			
Total	**6507.41**	**4680.77**	**100.9**	**2013.61**
Length percent	48.92	35.18	0.76	15.14

Table 5.6: An example of the raw data of severe preclamptic patient: PE27

	END(µm)	TPB(µm)	AV(µm)	NS(µm)
	215.42	76.59	36.17	143.5
	374.98	30.59	67.39	97.36
	22.94	179.92	44.28	30.72
	150.08	69.86	38.64	79.64
	61.87	156.35		7.28
	260.08	236.17		20.47
	164.2	25.91		19.6
	131.7	383.25		46.49
	347.1	104.87		26.51
	385.48	85.46		51.64
	64.99	83.84		172.22
	179.47	41.68		95.33
	42.74	69.36		127.61
	92.93	164.56		64.29
	156.94	174.91		95.86
	45.8	302.34		50.79
	52.5	168.36		118.94
	110.88	195.2		38.86
	41.68	314.13		117.63
	48.23	29.58		160.61
	90.72	100.57		51.26
	101.82	81.8		78.38
	55.19	13.74		170.07
	25.95	116.35		
	23.57	302.23		
	62.9	46.04		
	324.41	85.44		
	31.52	99.17		
	165.88	69.28		
	70.2	31.42		
	290.4	70.52		
		46.08		
Total	**4192.57**	**3955.57**	**186.48**	**1865.06**
Length Percent	41.1	38.78	1.83	18.29

Table 5.7: Length percent data for healthy control (1), mild pre-eclampsia (2) and severe pre-eclampsia (3) pateints

Patient code	Patient group	END(%)	TPB(%)	AV(%)	NS(%)
PC1	1	69.96	21.78	0.65	7.99
PC2	1	50.76	30.7	2.11	16.43
PC3	1	30.68	59.04	2.69	7.59
PC7	1	73.29	14.21	3.07	9.43
PC8	1	63.49	28.71	4.89	2.91
PC9	1	59.41	31.38	4.89	4.32
PC10	1	86.8	7.98	0.66	4.56
PC11	1	78.46	15.72	3.69	2.13
PC12	1	47.61	44.11	6.16	2.12
PC13	1	55.08	35.98	5.44	3.5
PC002	1	75.57	14.41	5.82	4.2
PE4	2	49.41	41.71	4.5	4.38
PE7	2	76.81	13.21	0.28	9.7
PE8	2	53.54	31.76	5.04	9.66
PE10	2	67.36	21.4	2.61	8.63
PE15	2	90.53	7.07	1.14	1.26
PE17	2	73.46	14.94	0.28	11.32
PE19	2	68.47	26.51	0.93	4.09
PE23	2	78.87	11.36	1.43	8.34
PE25	2	48.92	35.18	0.76	15.14
PE002	2	90.5	5.16	2.5	1.84
PE2	3	50.99	38.47	2.52	8.02
PE6	3	74.49	7.96	2.41	15.14
PE9	3	72.16	16.92	1.38	9.54
PE11	3	78.85	20.03	0.27	0.85
PE14	3	17.57	63.21	5.93	13.29
PE21	3	64.13	26.1	2.11	7.66
PE26	3	73.45	20.66	1.24	4.65
PE28	3	60.94	34.84	0.27	3.95
PE27	3	41.1	38.78	1.83	18.29
PE20	3	12.25	60.66	4.99	22.1
PE22	3	17.55	61.98	1.62	18.85

END = Endothelium; TPB = trophoblast; AV = Anchoring villous;

NS = Fibrin/fibrinoid

Table 5.8: Transformed data

Patient group	END/TPB	NS-AV	AV/NS
1	3.212121	7.34	0.081352
1	1.65342	14.32	0.128424
1	0.519648	4.9	0.354414
1	5.157635	6.36	0.325557
1	2.211425	-1.98	1.680412
1	1.893244	-0.57	1.131944
1	10.877193	3.9	0.144737
1	4.991094	-1.56	1.732394
1	1.079347	-4.04	2.90566
1	1.53085	-1.94	1.554286
1	5.244275	-1.62	1.385714
2	1.184608	-0.12	1.027397
2	5.814534	9.42	0.028866
2	1.685768	4.62	0.521739
2	3.147664	6.02	0.302433
2	12.804809	0.12	0.904762
2	4.913712	11.04	0.024735
2	2.582799	3.16	0.227384
2	6.942782	6.91	0.171463
2	1.390563	14.38	0.050198
2	17.53876	-0.66	1.358696
3	1.325448	5.5	0.314214
3	9.35804	12.73	0.159181
3	4.264775	8.16	0.144654
3	3.936595	0.58	0.317647
3	0.277962	7.36	0.4462
3	2.457088	5.55	0.275457
3	3.555179	3.41	0.266667
3	1.749139	3.68	0.068354
3	1.059825	16.46	0.100055
3	0.201945	17.11	0.225792
3	0.283156	17.23	0.085942

END/TPB = endothelium to trophoblast ratio, NS-AV =Length percent fibrin minus length percent anchoring villi, AV/NS = ratio of length anchoring villi to length percent fibrin; 1= control. 2 = mild pre-eclampsia; 3 = severe pre-eclampsia

APPENDIX 5: STATISTICAL ANALYSIS

Histogram for control patients

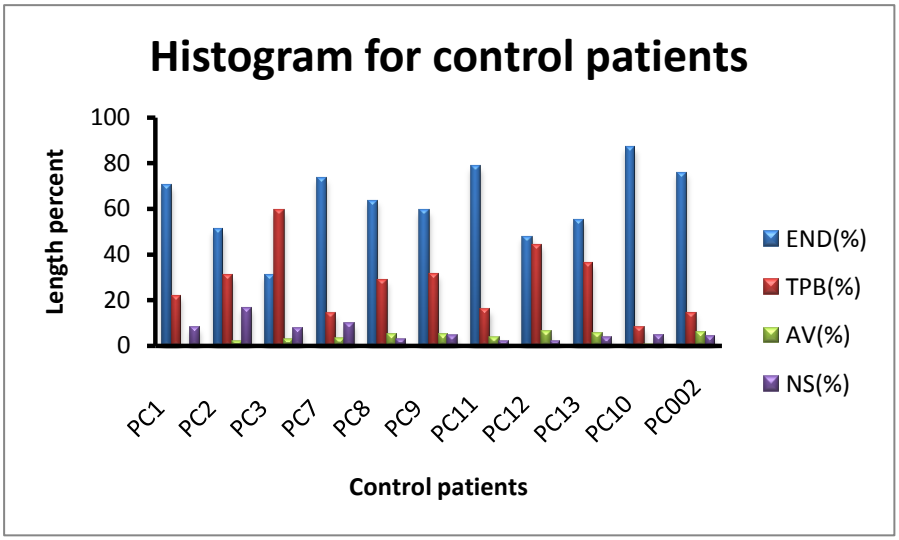

Fig. 5.1:A histogram of length proportion of the endothelium,trohpoblast and fibrin/fibrinoid lining the intervillous space border of the basal plate of healthy control patients placentae. The graph shows a higher proportion of endothelium than trophoblast and fibrin/fibrinoid except the placenta of patient PC3 in which there is more trophoblast than endothelium

Histogram for the mild pre-eclamptic patients

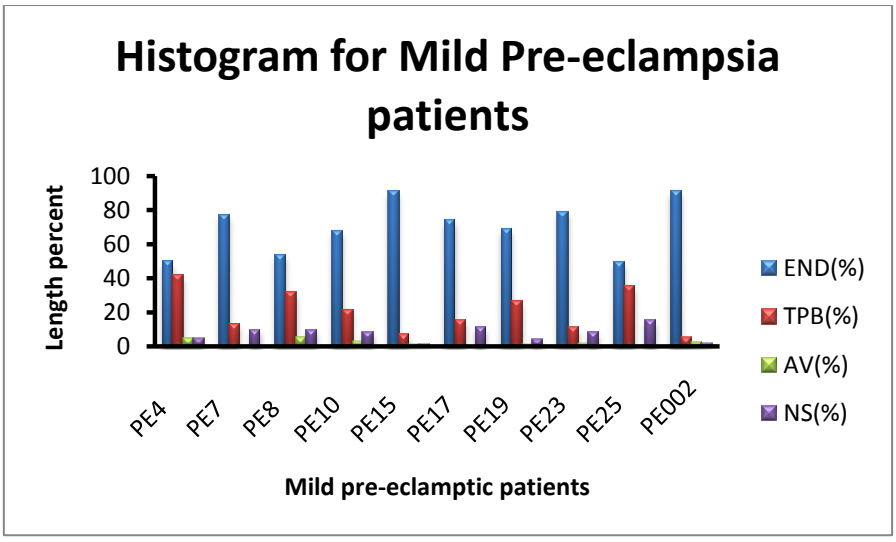

Fig. 5.2: A histogram of length percent of the endothelium, trophoblast and fibrin/fibrinoid lining the intervillous space border of the basal plate of placentae of mild pre-eclamptic patients. The graph shows that the proportion of the surface lined by endothelium is higher than that of trophoblast, and fibrin/fibrinoid. END= endothelium, TPB= trophoblast, NS= fibrin/fibrinoid.

Histogram for the Severe pre-eclamptic patients

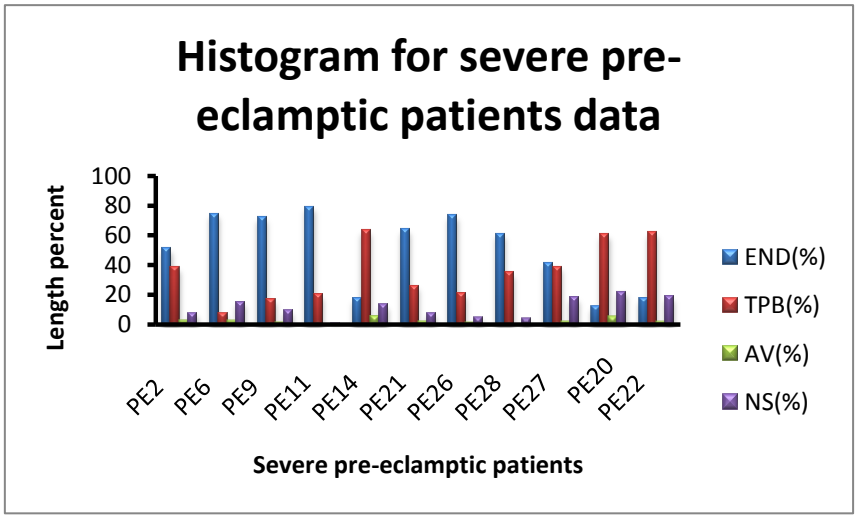

Fig. 5.3*:* A histogram of length percent of the endothelium, trophoblast and fibrin/fibrinoid lining the intervillous space border of the basal plate of placentae of severe pre-eclamptic patients. The graph shows that the proportion of the surface lined by endothelium is higher than that of trophoblast, and fibrin/fibrinoid except for three patiect placentae (PE14, PE20, and PE22) where trophoblast lined a larger proportion to the basal plate linig. END= endothelium, TPB= trophoblast, NS= fibrin/fibrinoid.

Table 5.9: Test of normality of the demographic data of the study groups

Tests of Normality

	Patient group	Test					
		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistics			Statistics		
		Statistic	df	Sig.	Statistic	df	Sig.
maternal age(years)	Healthy Control	.191	11	.200*	.908	11	.233
	Mild pre-eclamptic	.206	10	.200*	.823	10	.028
	Severe pre-eclamptic	.219	11	.146	.912	11	.259
Gestational age at delivery(weeks)	Healthy Control	.266	11	.029	.719	11	.001
	Mild pre-eclamptic	.178	10	.200*	.907	10	.258
	Severe pre-eclamptic	.164	11	.200*	.953	11	.684
Birthweight (grams)	Healthy Control	.135	11	.200*	.979	11	.958
	Mild pre-eclamptic	.245	10	.090	.883	10	.140
	Severe pre-eclamptic	.193	11	.200*	.926	11	.374

a. Lilliefors Significance Correction

*. This is a lower bound of the true significance.

This is a table showing the test of normality for maternal age, gestational age at delivery and the birth weight for the study groups. The test is not significant in all but for gestational age distribution in the healthy control group. The distribution of the data therefore fits normal distribution in all but the healthy control group. The test statistics employed was ANOVA.

Table 5.10: Oneway ANOVA result of the clinical characteristics of the groups.

ANOVA

Source=Between Groups

	Sum of Squares	df	Mean Square	F	Sig.
Maternal age(years)	87.784	2	43.892	1.967	.158
Gestational age at delivery(weeks)	104.557	2	52.278	9.576	.001
Birthweight (grams)	9182698.684	2	4591349.342	11.912	.000
Sex of the baby	.202	2	.101	.382	.686

This is a table of the test statistic result of the between groups analysis of variance. The means of gestational age at delivery and the birth weight are significantly different between the groups (P = 0.001 for GAD and p = 0.000 for birthweight). Means of maternal ages are not significantly different between the groups.

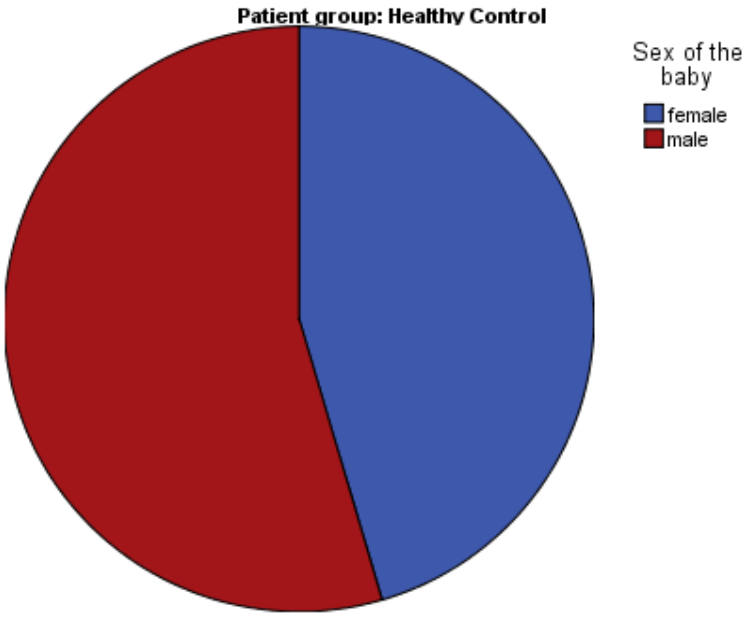

Fig. 5.4: A pie chart showing sex of the babies born to healthy mothers in the study population.

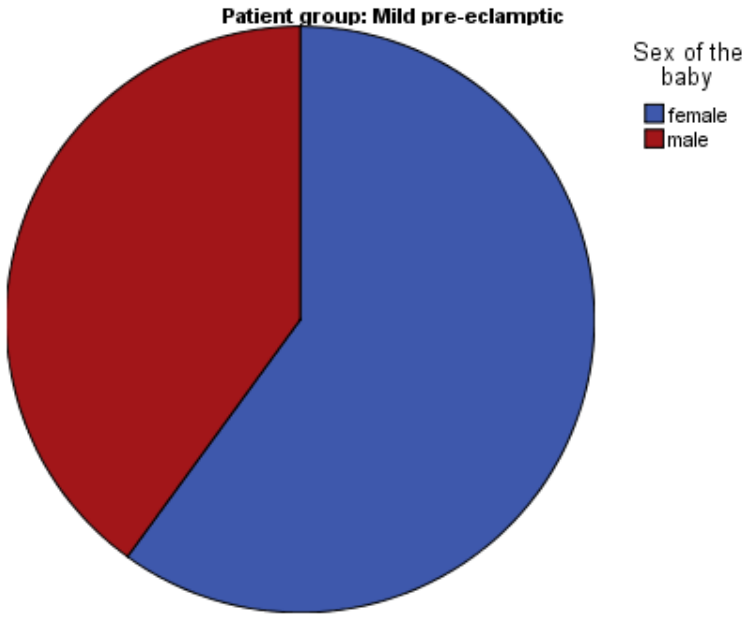

Fig. 5.5: Sex distribution of the babies born to mothers who suffered mild pre-eclampsia during pregnancy in the study population.

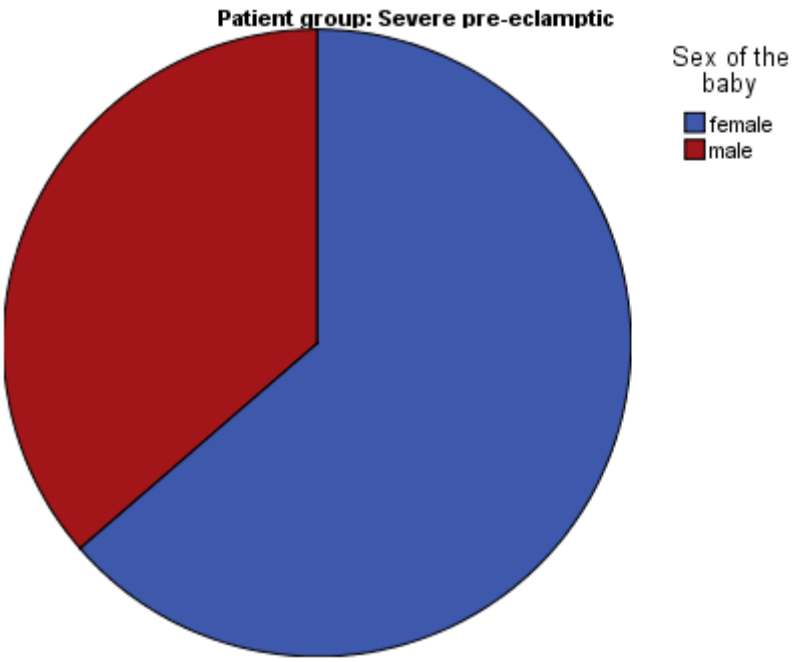

Fig. 5.6: Sex distribution of the babies born to mothers whose pregnancies were complicated by severe pre-eclampsia in the study population.

Table 5.11: Descriptive statistics of the length percent endothelium data for the patient groups.

Descriptives

Dependent Variables=Length percent endothelium

Statistics		Statistic			Std. Error		
		Patient group			Patient group		
		Healthy Control	Mild pre-eclamptic	Severe pre-eclamptic	Healthy Control	Mild pre-eclamptic	Severe pre-eclamptic
Mean		62.8282	69.7870	51.2255	4.88464	4.86125	7.61925
95% Confidence Interval for Mean	Lower Bound	51.9445	58.7901	34.2487			
	Upper Bound	73.7118	80.7839	68.2022			
5% Trimmed Mean		63.2824	69.7939	51.8561			
Median		63.4900	70.9650	60.9400			
Variance		262.456	236.318	638.583			
Std. Deviation		16.20051	15.37263	25.27020			
Minimum		30.68	48.92	12.25			
Maximum		86.80	90.53	78.85			
Range		56.12	41.61	66.60			
Interquartile Range		24.81	29.27	55.88			
Skewness		-.513	-.111	-.603	.661	.687	.661
Kurtosis		.032	-1.172	-1.363	1.279	1.334	1.279

It shows the means and trimmed means are almost equal for each group and Skewness and kurtosis are close to 1, indicating the data fits a normal distribution for the groups

Table 5.12: Descriptive statistics of the length percent trophoblast within the groups.

Descriptives

Dependent Variables=Length percent trophoblast

Statistics		Statistic			Std. Error		
		Patient group			Patient group		
		Healthy Control	Mild pre-eclamptic	Severe pre-eclamptic	Healthy Control	Mild pre-eclamptic	Severe pre-eclamptic
Mean		27.6382	20.8310	35.4191	4.54234	3.95974	5.85707
95% Confidence Interval for Mean	Lower Bound	17.5172	11.8735	22.3687			
	Upper Bound	37.7591	29.7885	48.4695			
5% Trimmed Mean		26.9858	20.5417	35.4007			
Median		28.7100	18.1750	34.8400			
Variance		226.961	156.795	377.358			
Std. Deviation		15.06523	12.52178	19.42570			
Minimum		7.98	5.16	7.96			
Maximum		59.04	41.71	63.21			
Range		51.06	36.55	55.25			
Interquartile Range		21.57	22.33	40.63			
Skewness		.782	.396	.361	.661	.687	.661
Kurtosis		.406	-1.174	-1.209	1.279	1.334	1.279

Tthe means and trimmed means are almost equal for each group and Skewness and kurtosis are close to 1, indicating the data fits a normal distribution for the groups.

Table 5.13: Descriptive statistics of length percent of anchoring villi within the study groups.

Descriptives

Dependent Variables=Length percent anchoring villi

Statistics		Statistic			Std. Error		
		Patient group			Patient group		
		Healthy Control	Mild pre-eclamptic	Severe pre-eclamptic	Healthy Control	Mild pre-eclamptic	Severe pre-eclamptic
Mean		3.6427	1.9470	2.2336	.59436	.53489	.53391
95% Confidence Interval for Mean	Lower Bound	2.3184	.7370	1.0440			
	Upper Bound	4.9670	3.1570	3.4233			
5% Trimmed Mean		3.6691	1.8678	2.1374			
Median		3.6900	1.2850	1.8300			
Variance		3.886	2.861	3.136			
Std. Deviation		1.97127	1.69146	1.77077			
Minimum		.65	.28	.27			
Maximum		6.16	5.04	5.93			
Range		5.51	4.76	5.66			
Interquartile Range		3.33	2.44	1.28			
Skewness		-.352	.977	1.198	.661	.687	.661
Kurtosis		-1.208	-.252	.976	1.279	1.334	1.279

It shows the means and trimmed means are almost equal for each group and Skewness and kurtosis are close to 1, indicating the data fits a normal distribution for the groups.

Table 5.14: Descriptive statistics table for length percent fibrin for the patients groups.

Descriptives

Dependent Variables=Length percent fibrin/fibrinoid

Statistics		Statistic			Std. Error		
		Patient group			Patient group		
		Healthy Control	Mild pre-eclamptic	Severe pre-eclamptic	Healthy Control	Mild pre-eclamptic	Severe pre-eclamptic
Mean		5.9255	7.4360	11.1218	1.28255	1.40137	2.07892
95% Confidence Interval for Mean	Lower Bound	3.0678	4.2659	6.4897			
	Upper Bound	8.7831	10.6061	15.7540			
5% Trimmed Mean		5.5533	7.3511	11.0826			
Median		4.3200	8.4850	9.5400			
Variance		18.094	19.638	47.541			
Std. Deviation		4.25373	4.43151	6.89501			
Minimum		2.12	1.26	.85			
Maximum		16.43	15.14	22.10			
Range		14.31	13.88	21.25			
Interquartile Range		5.08	6.58	13.64			
Skewness		1.671	.102	.160	.661	.687	.661
Kurtosis		3.060	-.688	-1.182	1.279	1.334	1.279

The table shows the means and trimmed means are almost equal for each group indicating that the data is normally distributed.

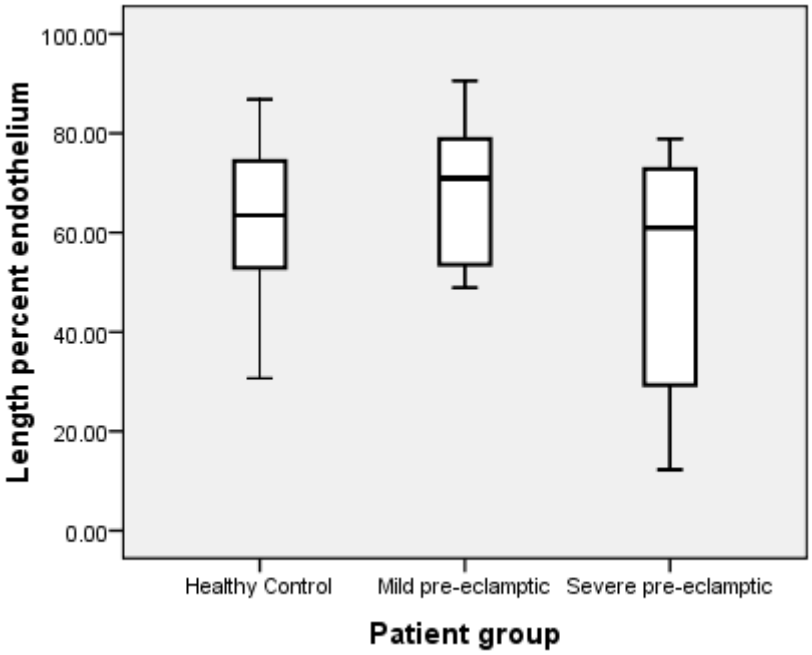

Fig. 5.7: Distribution of the length percent endothelium in the 3 patient groups showing mild pre-eclamptic patients have a larger median of endothelium (70.97) than those with healthy (63.49) and severe preclamptic (60.94) pregnancies.

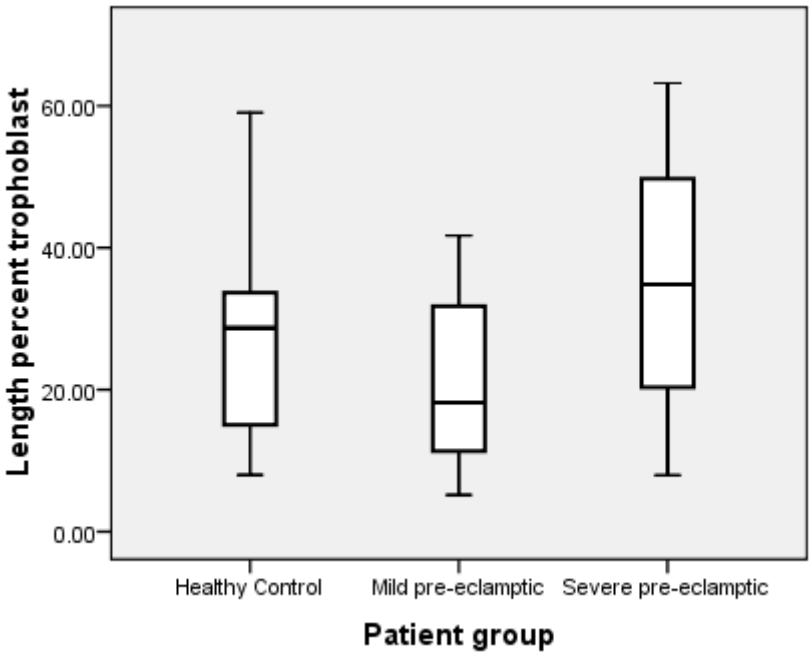

Fig. 5.8: Boxplot of length percent trophoblast against patient groups. The 50th percentile is represented by the thick line in the middle of the box. The ends of the whisker represent the lowest and the highest values for the groups.

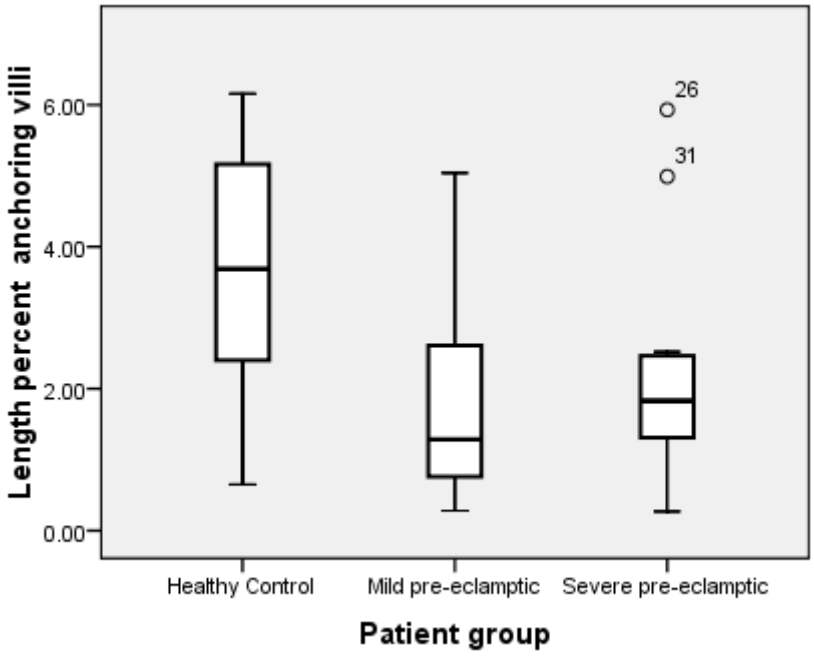

Fig. 5.9: Boxplot of length percent anchoring villi data for the study groups showing the median (line across the middle of the boxes) for each group, the range (the ends of the whiskers) and two outliers (o) for the severe pre-eclamptic patient data.

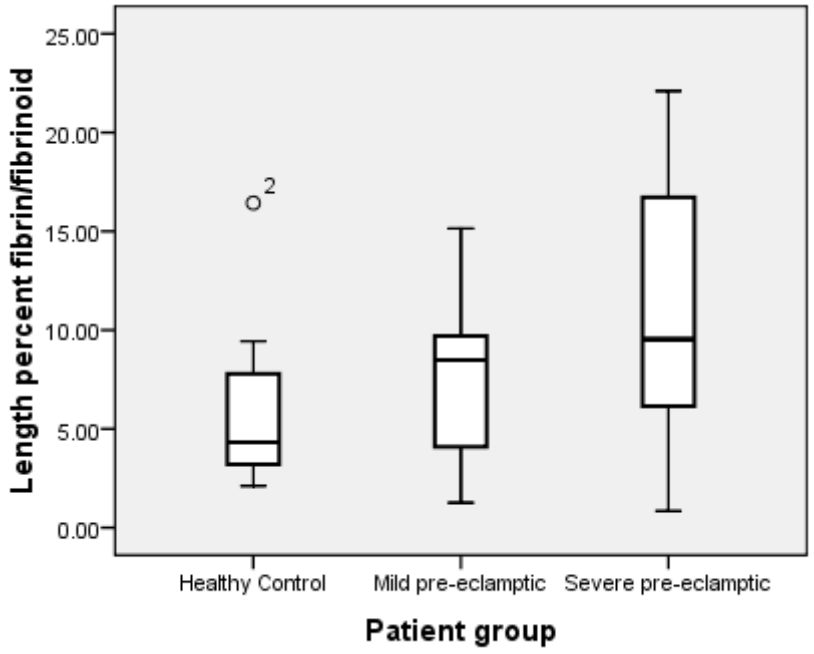

Fig. 5.10: Boxplot of length percent fibrin data for the study groups showing the median (line across the middle of the boxes) for each group, the range (the ends of the whiskers) and one outlier (o) for the control patient data.

Table 5.15: Test of normality of the measured data

Tests of Normality

Dependent Variables	Patient group	Kolmogorov-Smirnov[a] Statistics			Shapiro-Wilk Statistics		
		Statistic	df	Sig.	Statistic	df	Sig.
Length percent endothelium	Healthy Control	.125	11	.200*	.976	11	.939
	Mild pre-eclamptic	.155	10	.200*	.918	10	.338
	Severe pre-eclamptic	.195	11	.200*	.861	11	.060
Length percent trophoblast	Healthy Control	.149	11	.200*	.942	11	.549
	Mild pre-eclamptic	.181	10	.200*	.943	10	.588
	Severe pre-eclamptic	.176	11	.200*	.908	11	.228
Length percent anchoring villi	Healthy Control	.191	11	.200*	.924	11	.350
	Mild pre-eclamptic	.220	10	.186	.865	10	.088
	Severe pre-eclamptic	.254	11	.046	.864	11	.065
Length percent fibrin/fibrinoid	Healthy Control	.262	11	.033	.822	11	.018
	Mild pre-eclamptic	.181	10	.200*	.948	10	.645
	Severe pre-eclamptic	.136	11	.200*	.960	11	.770

a. Lilliefors Significance Correction

*. This is a lower bound of the true significance.

This is a table showing the results of Kolmogorov-Smirnov and Shapiro-Wilk's tests of normality for the length percent of the basal plate component data for each group. The test is not significant for all the components in all the groups except the length percent fibrin data for the control. Hence the distribution of the endothelial, trophoblast and anchoring villi data fit normal distribution. The fibrin data is normally distributed for the mild and severe pre-eclamptics but is asymmetrically distributed (highlighted) in the control patient group.

Table 5.16: Comparison of the means of the length percent of the basal plate components between the groups

ANOVA

Source=Between Groups

Dependent Variable	Sum of Squares	df	Mean Square	F	Sig.
Length percent endothelium	1859.831	2	929.915	2.421	.107
Length percent trophoblast	1119.755	2	559.878	2.178	.131
Length percent anchoring villi	17.675	2	8.837	2.671	.086
Length percent fibrin/fibrinoid	156.645	2	78.322	2.726	.082

Analysis of variance results of the length percent of the basal plate components. It shows that the differences between the means of the length percent of the components are not statistically significant between the groups as the p values (highlighted column) are > 0.05.

Table 5.17: Descriptive statistics of difference between length percent fibrin and length percent anchoring villi.

Dependent Variables=Difference between Length percent fibrin and length percent anchoring villi

		Statistic			Std. Error		
		Patient group			Patient group		
		Healthy Control	Mild pre-eclamptic	Severe pre-eclamptic	Healthy Control	Mild pre-eclamptic	Severe pre-eclamptic
Mean		2.2827	5.4890	8.8882	1.68058	1.60518	1.81139
95% Confidence Interval for Mean	Lower Bound	-1.4618	1.8578	4.8521			
	Upper Bound	6.0273	9.1202	12.9242			
5% Trimmed Mean		1.9653	5.3367	8.8863			
Median		-.5700	5.3200	7.3600			
Variance		31.068	25.766	36.093			
Std. Deviation		5.57384	5.07602	6.00772			
Interquartile Range		8.30	9.76	12.78			
Skewness		1.001	.383	.376	.661	.687	.661
Kurtosis		.533	-.815	-1.411	1.279	1.334	1.279

Table 5.18: Table of descriptive statistics of length percent anchoring villi to length percent of fibrin ratio within the patient groups showing the means and trimmed mean are about equal indicating that the transformed data fits normal distribution.

Dependent Variables=length percent anchoring villi to length percent fibrin ratio

		Statistic			Std. Error		
		Patient group			Patient group		
		Healthy Control	Mild pre-eclamptic	Severe pre-eclamptic	Healthy Control	Mild pre-eclamptic	Severe pre-eclamptic
Mean		1.0386	.4618	.2186	.27460	.15043	.03562
95% Confidence Interval for Mean	Lower Bound	.4268	.1215	.1392			
	Upper Bound	1.6505	.8021	.2979			
5% Trimmed Mean		.9881	.4362	.2143			
Median		1.1319	.2649	.2258			
Variance		.829	.226	.014			
Std. Deviation		.91075	.47570	.11814			
Interquartile Range		1.54	.89	.21			
Skewness		.689	.921	.440	.661	.687	.661
Kurtosis		-.095	-.507	-.460	1.279	1.334	1.279

Table 5.19: Table of descriptive statistics of length percent endothelium to length percent of trophoblast ratios within the patient groups showing the means and trimmed means are about 0.2- 0.4 different for each group. Skewness and Kurtosis are also greater than 1 indicating that the data may not be normally distributed

		Stat Type					
		Statistic			Std. Error		
		Patient group			Patient group		
Statistics		Healthy Control	Mild pre-eclamptic	Severe pre-eclamptic	Healthy Control	Mild pre-eclamptic	Severe pre-eclamptic
Mean		3.4882	5.8006	2.5881	.89843	1.71275	.81123
95% Confidence Interval for Mean	Lower Bound	1.4864	1.9261	.7806			
	Upper Bound	5.4900	9.6751	4.3956			
5% Trimmed Mean		3.2426	5.4049	2.3446			
Median		2.2114	4.0307	1.7491			
Variance		8.879	29.335	7.239			
Std. Deviation		2.97976	5.41618	2.69056			
Interquartile Range		3.63	6.80	3.65			
Skewness		1.649	1.448	1.719	.661	.687	.661
Kurtosis		3.158	1.410	3.566	1.279	1.334	1.279

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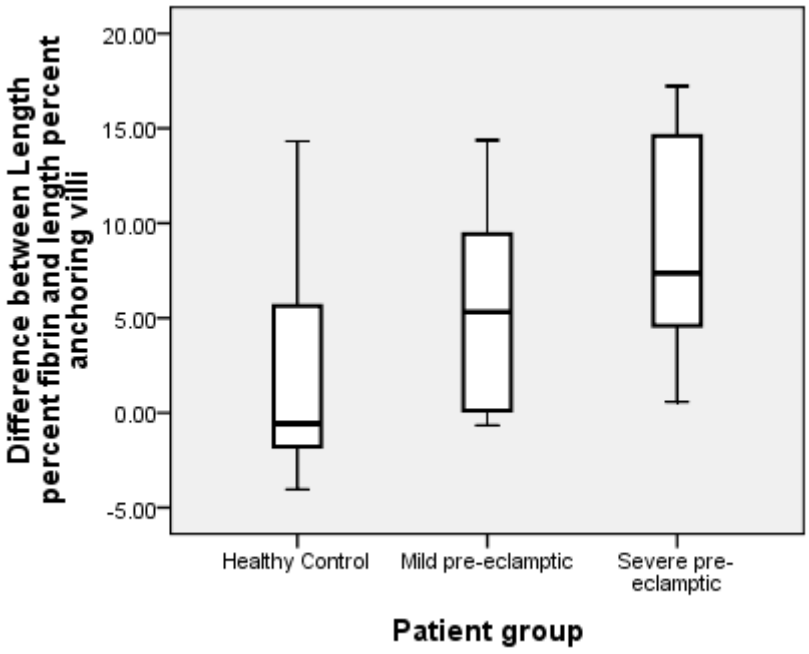

Fig. 5.11: A box plot of the NS-AV data against the patient groups showing an increase in the median of the NS-AV with increasing severity of pre-eclampsia.

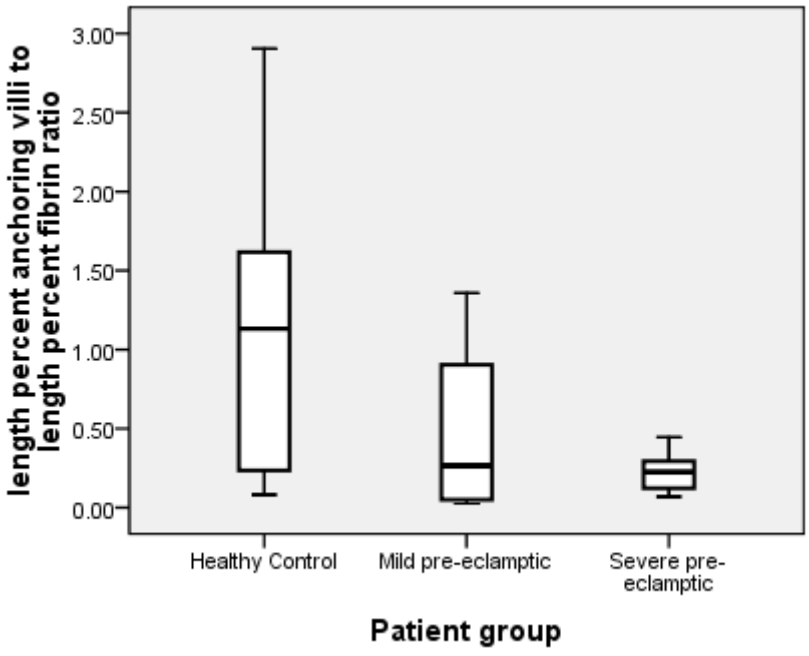

Fig. 5.12: A box plot of AV to NS ratio in the three patient groups showing a lower median of the ratio in the mild and severe pre-eclamptic groups than the control group.

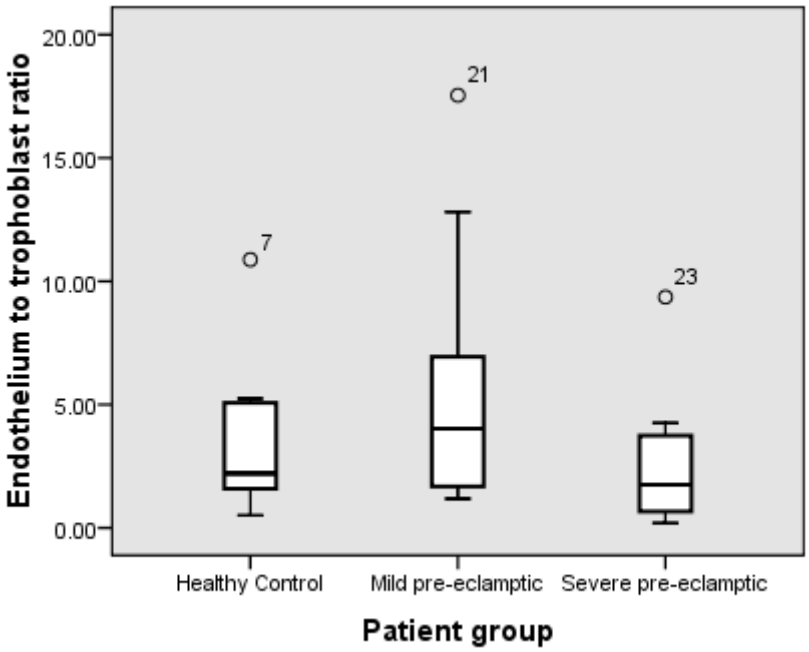

Fig. 5.13: A box plot showing the distribution of the END to TPB ratio in three patient groups. The median is slightly higher in the mild pre-eclamptic patients than the mild pre-eclamptic and the healthy controls. It also shows one outlier each for each group.

Table 5.20: Test of normality for the transformed data

Tests of Normality

	Patient group	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Difference between Length percent fibrin and length percent anchoring villi	Healthy Control	.241	11	.074	.883	11	.114
	Mild pre-eclamptic	.155	10	.200*	.946	10	.620
	Severe pre-eclamptic	.185	11	.200*	.895	11	.160
length percent anchoring villi to length percent fibrin ratio	Healthy Control	.228	11	.114	.884	11	.118
	Mild pre-eclamptic	.231	10	.139	.859	10	.075
	Severe pre-eclamptic	.147	11	.200*	.944	11	.573
Endothelium to trophoblast ratio	Healthy Control	.211	11	.183	.827	11	.022
	Mild pre-eclamptic	.216	10	.200*	.822	10	.027
	Severe pre-eclamptic	.188	11	.200*	.821	11	.018

a. Lilliefors Significance Correction

*. This is a lower bound of the true significance.

This is a table showing the results of Kolmogorov-Smirnov and Shapiro-Wilk tests of normality for the transformed data (NS-AV, AV to NS ratio and END to TPB ratio) for each group. The K-S test is not significant for all the data in all the groups. The Shapiro-Wilk test is not significant for the NS-AV and AV to NS ratio in all the groups. It is however significant for END to TPB ratio in all the groups. Hence the distribution of the NS-AV and AV to NS ratio data fit normal distribution. The END to TPB ratio data is not normally distributed.

Comparison of the means of the NS-AV and AV to NS ratio data between the groups

Since the data for the above are parametric, ANOVA was performed.

Table 5.21: Test of homogeneity for the length percent fibrin minus the length percent trophoblast (NS-AV) and the ratio of length percent anchoring villi to length percent fibrin ratio (AV: NS).

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
Difference between Length percent fibrin and length percent anchoring villi	.362	2	29	.699
length percent anchoring villi to length percent fibrin ratio	13.715	2	29	.000

The results show that the variation of the mean for the AV: NS is not equal across the groups as test is not significant ($p < 0.000$). This violates the assumption of ANOVA but ANOVA is robust against this violation. The mean variance for the NS- AV data is equal across the group as the $p = 0.699$.

Table 5.22: Analysis of variance for NS-AV and AV: NS ratio

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Difference between Length percent fibrin and length percent anchoring villi	Between Groups	240.040	2	120.020	3.852	.033
	Within Groups	903.497	29	31.155		
	Total	1143.538	31			
length percent anchoring villi to length percent fibrin ratio	Between Groups	3.890	2	1.945	5.387	.010
	Within Groups	10.471	29	.361		
	Total	14.361	31			

ANOVA results shows that the means of the data are significantly different between the control, mild and severe pre-eclmapsia groups as indicated by the p values (highlighted) < 0.05.

PostHoc comparison of the means of the three groups

Table 5.23: Patient group=Severe pre-eclamptic

Multiple Comparisons

(J) Patient group	Dependent Variable	Tukey HSD				
		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Healthy Control	Difference between Length percent fibrin and length percent anchoring villi	6.60545*	2.38003	.025	.7276	12.4833
	length percent anchoring villi to length percent fibrin ratio	-.82007*	.25622	.009	-1.4528	-.1873
Mild pre-eclamptic	Difference between Length percent fibrin and length percent anchoring villi	3.39918	2.43881	.357	-2.6238	9.4222
	length percent anchoring villi to length percent fibrin ratio	-.24321	.26255	.628	-.8916	.4052

*. The mean difference is significant at the 0.05 level.

Multiple comparison of the severe pre-eclamptic group to the control and the mild pre-eclamptic patients shows that the means of the AV to NS ratio and NS-AV for the severe pre-eclamptics are significantly different from the healthy control group (p= 0.025 and p=0.009). The means of the severe patient group is not different from the mild patient group (p=0.357 and 0.628)

Table 5.24: Patient group=Mild pre-eclamptic

Multiple Comparisons

(J) Patient group	Dependent Variable	Tukey HSD				
		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Healthy Control	Difference between Length percent fibrin and length percent anchoring villi	3.20627	2.43881	.399	-2.8167	9.2293
	length percent anchoring villi to length percent fibrin ratio	-.57686	.26255	.089	-1.2253	.0715
Severe pre-eclamptic	Difference between Length percent fibrin and length percent anchoring villi	-3.39918	2.43881	.357	-9.4222	2.6238
	length percent anchoring villi to length percent fibrin ratio	.24321	.26255	.628	-.4052	.8916

Comparing the means of the NS-AV data and the AV to NS ratio data of the mild group to the control and the severe patients groups show that there is no difference between the means as the p values are greater than 0.05.

Non-parametric analysis of the Endothelium to trophoblast ratio

Since the distribution of the END to TPB ratio does not fit a normal distribution a nonparametric analysis was applied for this data.

Table 5.25 A and B: Nonparametric analysis of endothelium to trophoblast ratio

Ranks

	Patient group	N	Mean Rank
Endothelium to trophoblast ratio	Healthy Control	11	16.73
	Mild pre-eclamptic	10	20.40
	Severe pre-eclamptic	11	12.73
	Total	32	

A

Test Statistics[a,b]

	Endothelium to trophoblast ratio
Chi-Square	3.514
df	2
Asymp. Sig.	.173

B

a. Kruskal Wallis Test

b. Grouping Variable: Patient group

This is the result of the Kruskal-Wallis test of the END to TPB ratio. It shows that the median of the END to TPB ratio data is not significantly different between the three patient groups as the p value of 0.173 > 0.05.

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