
بسم الله الرحمن الرحيم

STUDY OF SERUM LEVEL OF UROTENSIN II

IN FEMALES WITH PRE-ECLAMPTIC TOXAEMIA OF PREGNANCY

By

Nesrin Mohamed Thabet Mahran

SUPERVISORS

Prof. Dr. Mona Mohamed Abaza

Professor of Chemical Pathology
Medical Research Institute
Alexandria University

Dr. Gehan Mahmoud Hamdy Magour

Assistant Professor of Chemical Pathology
Medical Research Institute
Alexandria University

Dr. Mervat Sheikh El-Arab El- Sedeek

Assistant Professor of Obstetrics and Gynaecology
Faculty of Medicine
Alexandria University

Dr. Hisham Salah El-Din El Banawy

Lecturer of Chemical Pathology
Medical Research Institute
Alexandria University

The placenta

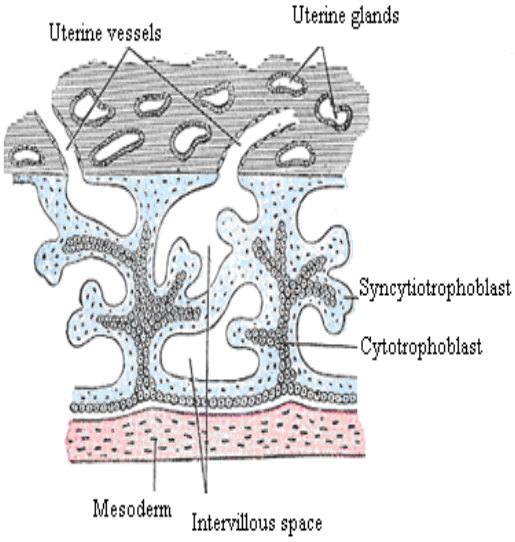
The placenta (Greek, *plakuos* = flat cake) is a temporary feto-maternal organ within the pregnant uterus and delivered with the fetus at birth. It anchors the developing embryo to the uterus and provides a bridge for the exchange of nutrients, oxygen, protective antibodies and waste products between the fetus and the mother. [1]

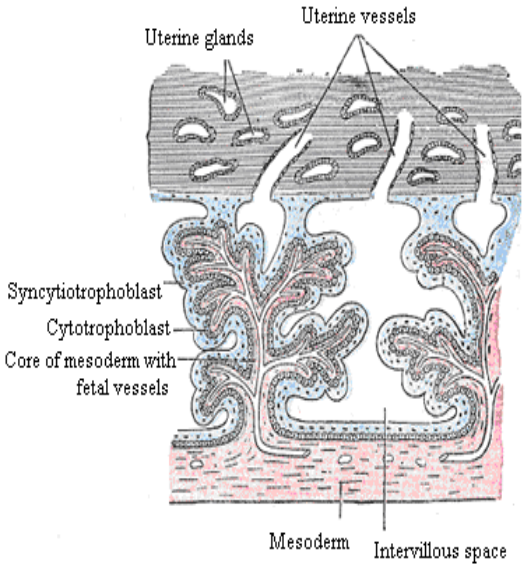
The placenta is composed of three layers: [2]

- The innermost placental layer surrounding the fetus is called the amnion.
- The middle layer is the allantois in which the blood vessels originating from the umbilicus traverse across it.
- The outermost layer of the placenta; the chorion, comes into contact with the endometrium. It is composed of two layers of cells; the outer syncytiotrophoblast and the inner cytotrophoblast. The chorion and allantois fuse to form the chorioallantoic membrane.

Chorionic villi [3]

The chorion undergoes rapid proliferation and forms numerous finger-like projections on its surface (chorionic villi). The chorionic villi are at first small and non-vascular, and consist of trophoblast only, then they increase in size and ramify. Blood is carried to the villi by the branches of the umbilical arteries, and after circulating through the capillaries of the villi, is returned to the embryo by the umbilical veins. (Figure 1)[4]





Primery chorionic villi. *Secondary chorionic villi.*

Figure (1): chorionic villi. [4]

The Decidua. (5)

Before the fertilized ovum reaches the uterus, the mucous membrane of the body of the uterus undergoes important changes and is then known as the decidua**.** The thickness and vascularity of the mucous membrane are greatly increased; its glands are elongated and open on its free surface by funnel-shaped orifices, while their deeper portions are tortuous and dilated into irregular spaces (Figure 2)(4). The interglandular tissue is also increased in quantity, and is crowded with large round, oval, or polygonal cells, termed decidual cells**.**

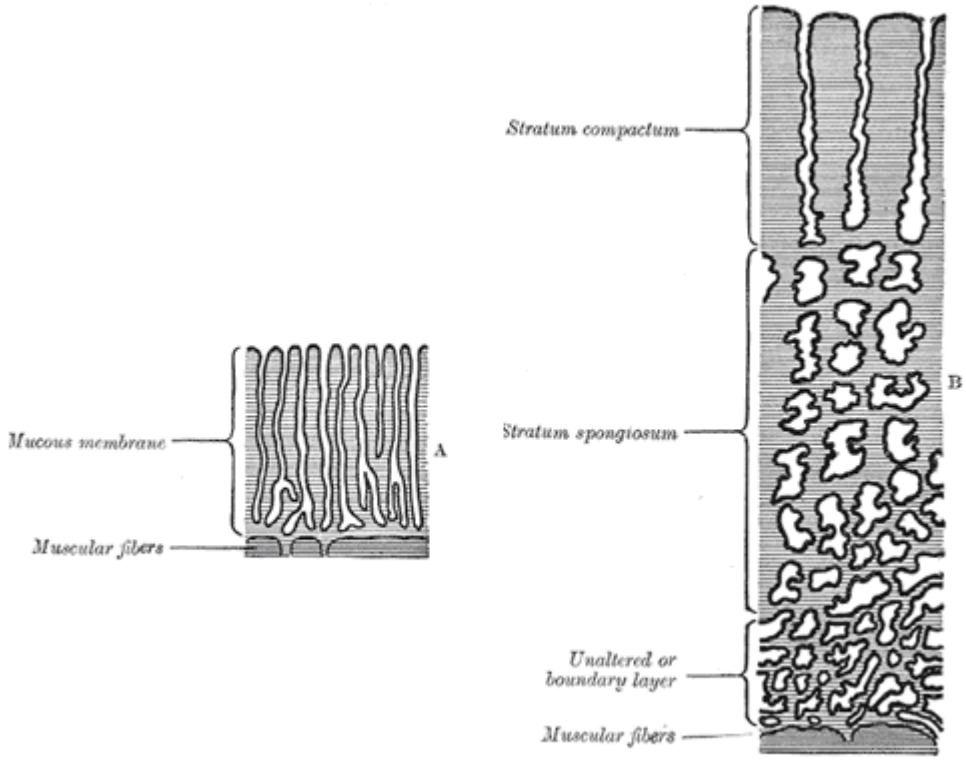


Figure (2): Diagrammatic sections of uterine mucous membrane:
A. non-pregnant uterus. B. the pregnant uterus, showing the thickened mucous membrane and altered condition of the uterine glands. (4)

Placental Blood vessels [6]

They were formed initially in the connecting stalk (then umbilical cord) and anastomose in the chorion.They extend maternally toward the chorionic villi and embryonically to the sinus venosus and dorsal aorta. (Figure 3)[4]

The arteries are paired and carry deoxygenated blood (from dorsal aorta) and waste products to the placental villi.

The veins are paired initially then only left at end of embryonic period and carry oxygenated blood to the embryo (sinus venosus).

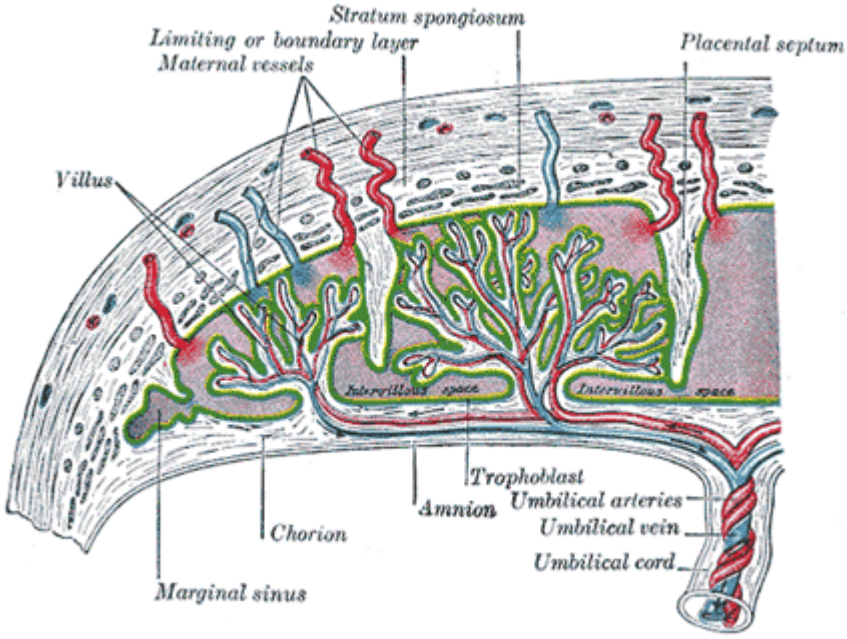


Figure (3): Placental blood vessels [4]

Feto-placental circulation [7]

Deoxygenated fetal blood passes through umbilical arteries to the placenta. At the junction of umbilical cord and placenta, the umbilical arteries branch to form chorionic arteries. Chorionic arteries also branch before they enter into the villi. In the villi, they form an extensive arteriocapillary venous system, bringing the fetal blood extremely close to the maternal blood; but normally no intermingling of fetal and maternal blood occurs.

Placental Functions

- Placental metabolic functions [8]

- Synthesizes the glycogen, cholesterol and fatty acids required by the fetus.
- Provides nutrients and energy to the fetus.

- Placental transport functions [9]

- The placenta receives nutrients, oxygen, antibodies and hormones from the mother's blood transporting them to fetus, and passes out waste.
- It forms a barrier; the placental barrier, which filters out some substances that could harm the fetus. Many substances are not filtered out, including alcohol, all anesthesia drugs used in medical childbirth (opoids and cocaine derivatives), and some chemicals associated with smoking cigarettes. Several types of viruses, such as human cytomegalovirus, may also cross this barrier; this often leads to various degrees of birth defects in the infant.

- Placental endocrinal functions [10]

The placenta produces hormones as:

- Human chorionic gonadotrophin which supports corpus luteum.
- Human chorionic somatomamotropin(also known as placental lactogen), which stimulates mammary gland development and increases the amount of glucose and lipids in the maternal blood.
- Progesterone which supports maternal endometrium and is important in maintaining the pregnancy.

- Placental immunologic functions [11]

Since the fetus contains genetic material from both the mother and the father it is said to be semi-allogenic for the mother, this means that the mother and fetus would have different major histocompatability complexes (MHC).

The mother's blood (with immunocompetent lymphocytes) is in direct contact with fetal trophoblast, so there has to be a mechanism to stop the fetus being rejected. The most important factor is the lack of both class I and class II MHC antigens on the chorionic villi, thus giving the fetus protection against allogenic attack by the mothers' immune system. However it has been found that some populations of cytotrophoblast cells produce a unique class I MHC molecule that has been named HLA G which has an integral part of the defence mechanism of the feto-placental unit.

Separation of the placenta.

The placenta is expelled from the uterus after the birth of fetus. The separation of the placenta from the uterine wall takes place through the stratum spongiosum, and necessarily causes rupture of the uterine vessels. The orifices of the torn vessels are closed by the firm contraction of the uterine muscular fibers, and thus postpartum hemorrhage is controlled. The epithelial lining of the uterus is regenerated by the proliferation and extension of the epithelium which lines the persistent portions of the uterine glands in the unaltered layer of the decidua. [12]

Pre-eclampsia

Pre-eclampsia is a serious materno-fetal disease that is diagnosed after 20 weeks of gestation on the basis of new onset of hypertension (blood pressure ≥140/90 mmHg) and proteinuria (at least 300 mg/24 hours urine). [13-14]

Epidemiology [15-16]

Pre-eclampsia is a worldwide disease that affects more than four million pregnant women each year. It complicates 6% of pregnancies, and is much more common in women who are pregnant for the first time. Approximately 2% of women with pre-eclampsia may pass into eclamptic convulsions.

Risk Factors [17-18]

Pre-eclampsia is more common in women who are old, have pre-existing hypertension, diabetes, autoimmune diseases, various inherited thrombophilias, renal disease, a history of pre-eclampsia in a previous pregnancy and with multiple gestation (twins, triplets and more).

Classification [19-20]

For the diagnosis of pre-eclampsia both hypertension and proteinuria must be present.
Pre-eclampsia is classified into mild and severe:

Mild pre-eclampsia:

- Blood pressure: systolic 140 mm Hg or higher and diastolic 90 mm Hg or higher after 20 weeks of gestation in a woman with previously normal blood pressure.
- Proteinuria: 0.3 gm or more of protein in 24-hours urine (1+ or greater on a urine dipstick test).

Severe pre-eclampsia:

- Blood pressure: systolic 160 mm Hg or higher and diastolic 110 mm Hg or higher on two measurements at least six hours apart in a woman on bed rest.
- Proteinuria: 5 gm or more of protein in a 24-hours urine (3+ or greater on urine dipstick test) of two random urine samples collected at least four hours apart.

Other features that may accompany sever pre-eclampsia are: oliguria (less than 500 mL of urine in 24 hours), cerebral or visual disturbances, pulmonary edema or cyanosis, epigastric or right upper quadrant pain, impaired liver function, thrombocytopenia and intrauterine growth restriction.

Clinical Presentation

The clinical presentation of pre-eclampsia may be insidious or fulminant. Some women may be asymptomatic at the time they are found to have hypertension and proteinuria. In severe pre-eclampsia: visual disturbances, severe headache or upper abdominal pain. [21]

However 4 to 14 percent of women with pre-eclampsia present with superimposed syndrome of hemolysis, elevated liver enzymes and low platelets count (HELLP syndrome) which may be a variant of pre-eclampsia or a separate entity, but its development is associated with serious morbidity or mortality. (22)

Pre-eclampsia-eclampsia may develop before, during, or after delivery. Death associated with pre-eclampsia-eclampsia may be due to cerebrovascular complication, renal or hepatic failure, HELLP syndrome or other complications of hypertension. (23)

Diagnostic Evaluation

- History

As a part of the initial prenatal assessment, pregnant women should be questioned about potential risk factors for pre-eclampsia. They should be asked about their obstetric history, specifically the occurrence of hypertension or pre-eclampsia during previous pregnancies. A thorough medical history should be obtained to identify medical conditions that increase the risk for pre-eclampsia, including diabetes mellitus, hypertension, vascular and connective tissue disease and nephropathy. (24)

During prenatal visits after 20 weeks of gestation, pregnant women should be asked about specific symptoms, including visual disturbances, persistent headaches, epigastric or right upper quadrant pain, and increased edema. Questions about these symptoms are included in many standardized prenatal documentation forms. (25)

- Physical examination

Blood pressure should be measured at each prenatal visit. To ensure accurate readings, an appropriate-size blood pressure cuff should be used, and blood pressure should be measured after a bed rest period of 10 minutes or more. During the blood pressure measurement, the patient should be in an upright or left lateral recumbent position with the arm at the level of the heart. (26)

Fundal height should be measured at each prenatal visit because levels less than dates may indicate intrauterine growth retardation or oligohydramnios. These conditions may become apparent long before diagnostic criteria for pre-eclampsia are met. (27)

Increasing maternal facial edema and rapid weight gain also should be noted because fluid retention often is associated with pre-eclampsia. Although these symptoms (e.g., facial oedema, rapid weight gain) are not unique to pre-eclampsia, it is wise to follow affected patients for hypertension and proteinuria. Oedema involving the lower extremities frequently occurs during normal pregnancy and therefore is of less concern. (28)

- Laboratory evaluation

There is no single reliable, cost-effective screening test for pre-eclampsia. A baseline laboratory evaluation should be performed early in pregnancy in women who are at high risk for pre-eclampsia. Tests should include hepatic enzymes activities, platelet

count, and serum creatinine level and 24-hours urine collection for total protein measurement. [29]

Once the diagnosis of pre-eclampsia has been made, an expanded set of laboratory tests should be performed. In women who have pre-eclampsia with no suspected progression, all laboratory tests should be conducted weekly and if eclampsia is suspected, the tests should be repeated more frequently. [30]

Random urinary protein to creatinine ratio predicts the 24-hours urine total protein level and provides a faster, simplified method of estimating proteinuria, provided that urinary protein values are less than 1 gm in 24 hours. [31] The urinary protein to creatinine ratio is not sensitive enough to differentiate mild and severe pre-eclampsia if significant proteinuria exists. However, proteinuria is diagnosed when urine protein to creatinine ratio ≥30 mg /mmol creatinine in a single urine specimen. [32-33]

- Other studies [28]

A baseline sonogram of uterus should be considered at 25 to 28 weeks of gestation to evaluate fetal growth in pregnant women at high risk for pre-eclampsia.

In women who have already been diagnosed with pre-eclampsia, antepartum testing with a nonstress test (a Doppler time series recording of the heart beat of a fetus in utero simultaneous with the abdominal and/or uterine contractions of the pregnant woman), a biophysical profile (a prenatal ultrasound evaluation of fetal wellbeing) or both should be performed on a weekly basis starting at the time of diagnosis. If intrauterine growth retardation or oligohydramnios is suspected, the tests should be performed at least twice weekly, and delivery will be indicated if there are any signs of fetal distress.

Differential diagnosis [34-35]

- Chronic hypertension is defined by elevated blood pressure predates the pregnancy or if present 12 weeks after delivery.

- Pre-eclampsia is diagnosed when elevated blood pressure and proteinuria occur after 20 weeks of gestation.

- Pre-eclampsia superimposed on chronic hypertension is characterized by new onset of proteinuria (or by a sudden increase in the protein level if proteinuria is already present), an acute increase in the level of hypertension (assuming proteinuria already exists), or development of the syndrome of hemolysis, elevated liver enzymes and low platelets count (HELLP syndrome).

- Gestational hypertension is diagnosed when elevated blood pressure occurs without proteinuria, it develops after 20 weeks of gestation and blood pressure returns to normal within 12 weeks after delivery. One fourth of women with gestational hypertension develop proteinuria and thus progress to pre-eclampsia.

Pathophysiology

Although the exact cause of pre-eclampsia remains unclear, one of the most striking physiologic changes is intense systemic vasospasm which is responsible for decreased perfusion of all body organs. Perfusion also is diminished because of vascular heamoconcentration accompanying pregnancy. Many theories center on problems of placental implantation and the level of trophoblastic invasion. [36]

In addition, pre-eclampsia is accompanied by an exaggerated inflammatory response and inappropriate endothelial activation of the coagulation cascade resulting in microthrombi formation. Also it is currently thought that tissue factors are released from the ischemic placenta affecting endothelial cells widely throughout the maternal circulation, [36] resulting in occlusive spasm of arterioles leading to complications including:

- Central Nervous System irritability manifested by headaches, visual disturbance, hyperreflexia and ultimately convulsions. The etiology of this is more likely to be on the basis of vasospasm and hypoxia rather than cerebral oedema as was originally thought. Convulsions are not directly related to an elevation in blood pressure (as compared with hypertensive encephalopathy). [37-38]

- Generalized arterial vasospasm leading to decreased circulating blood volume with variable amount of tissue oedema. The systemic vascular resistance is increased leading to left ventricular strain. Consequently there may be left ventricular diastolic dysfunction. [39]

- Thrombocytopenia is present in one third of patients, and in severe cases platelet counts may fall rapidly. In addition, platelet dysfunction may be present. Severe cases may develop HELLP syndrome and disseminated intravascular coagulopathy. [40]

- Pulmonary involvement is uncommon until late in the course of the disease when pulmonary oedema and upper airway (especially laryngeal) oedema may occur. Pulmonary oedema occurs most frequently after delivery. [41]

- Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) may be elevated particularly in the HELLP syndrome possibly due to areas of necrosis or ischemia. Hepatic rupture is a rare, but often lethal complication. [29]

- Proteinuria; indicates glomerular involvement, probably on a vascular basis. Oliguria is more commonly due to hypovolaemia and decreased renal blood flow rather than primary renal pathology. Progress to acute renal failure is common, especially with HELLP syndrome. However, renal outcomes are generally good. [42]

- Reduced placental perfusion which results in a high prevalence of intrauterine growth retardation. There is also a high incidence of abruptio placenta and preterm labour. [43]

Pathogenesis of Pre-eclampsia (Figure 4) [44]

Pre-eclampsia is a disease of theories, where several theories exist. The underlying causes of pre-eclampsia can be divided into: abnormal placental development, predisposing maternal constitutional factors, oxidative stress, immune maladaptation and genetic susceptibility. [45].

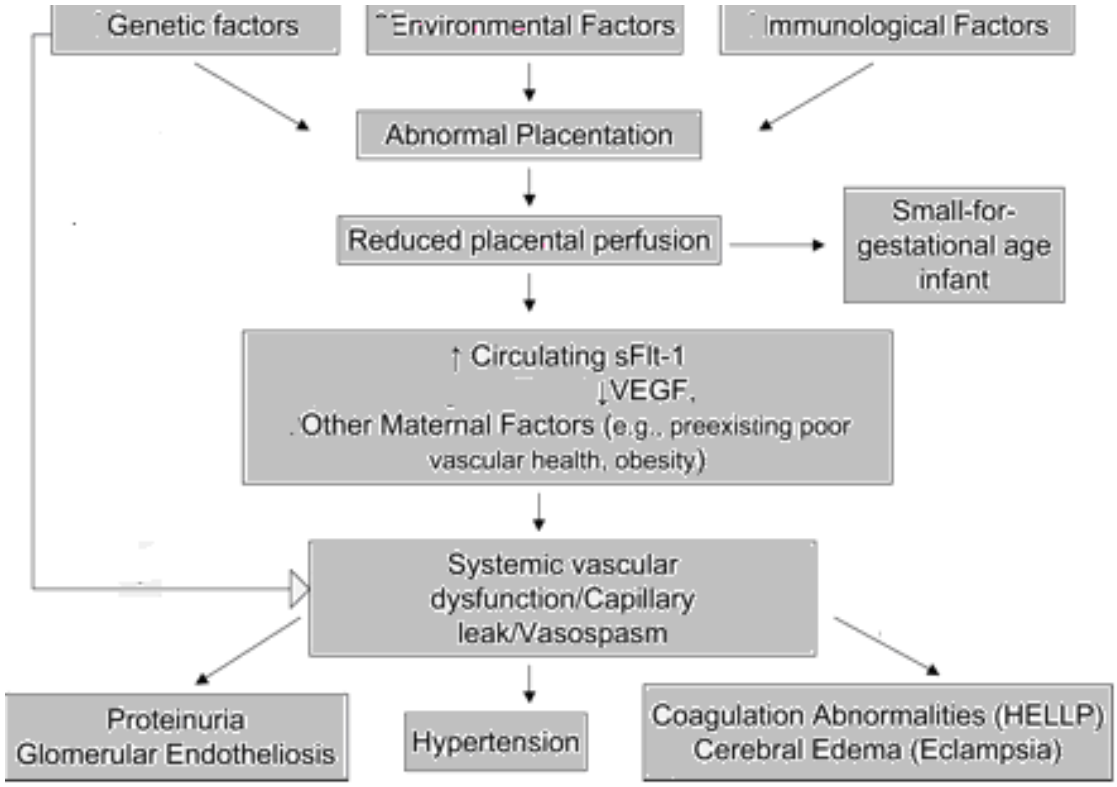


Figure (4): Summary of the pathogenesis of pre-eclampsia. [44]

Abnormal placental development (Figure 5) [44]

The placenta is a key source of factors that lead to maternal endothelial cell dysfunction in pre-eclampsia as the clinical and of pre-eclampsia remit within days after termination of pregnancy**.** [46]

On the basis of the observation that the only definitive cure for pre-eclampsia is delivery of the placenta and those women who experience a molar pregnancy, in which a placenta develops without a fetus, frequently develop severe pre-eclampsia, it is reasonable to assume that the placenta plays a central role in the pathogenesis of the disease. In rare cases of extrauterine pregnancy, in which delivery of the fetus is not followed by delivery of the placenta, the signs of pre-eclampsia persist after delivery until the placenta is resorbed. [25]

Early in normal gestation, the spiral arteries (the terminal branches of the uterine artery) are transformed from thick-walled, muscular vessels to sac-like vessels with decreased or lacking vasoconstrictive abilities. This transformation involves invasion of the spiral arteries by endovascular throphoblast cells of the placenta. [47]

In women who eventually develop pre-eclampsia, the invasion of the uterine spiral arteries is incomplete and shallow. At a morphological level, the vessels remaining thick-walled and muscular and the arterial component is rudimentary leading to hypoperfusion of the placenta. [48] At a molecular level, these defects are associated with particular deficits in the differentiation process whereby cytotrophoblasts assume vascular-like properties, and the placenta produces of vasoactive substances that reach the maternal circulation with the potential to produce at least a subset of the clinical signs of this syndrome. [49]

However this defective placentation may not be present in all pre-eclamptic women and severe placental ischemia does not necessarily lead to pre-eclampsia. [50]

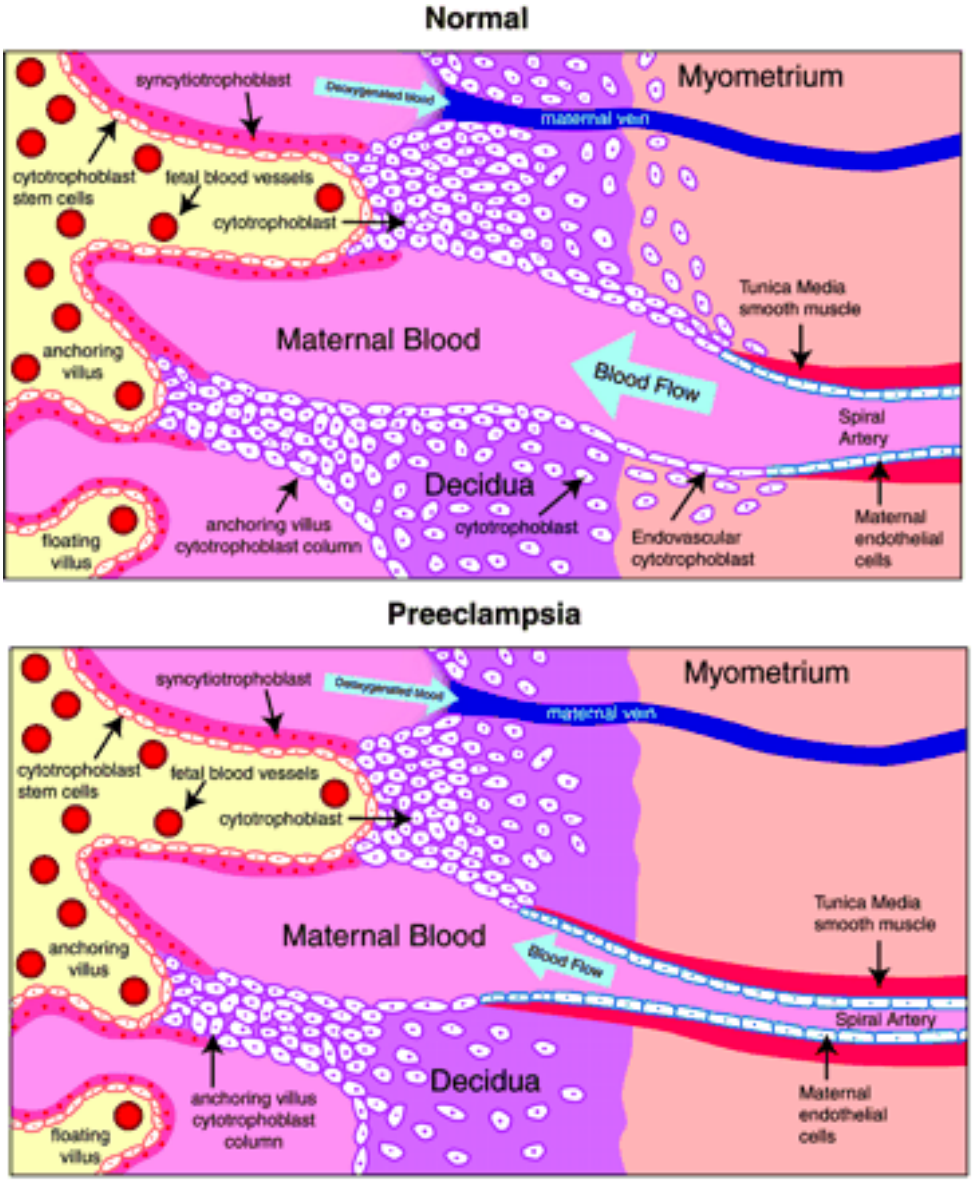


Figure (5)[(44)]: Abnormal placentation in pre-eclampsia.

Exchange of oxygen, nutrients, and waste products between the fetus and the mother depend on adequate placental perfusion by maternal vessels. In normal placental development, invasive cytotrophoblasts of fetal origin invade the maternal spiral arteries transforming them from small calibered resistance vessels to large calibered capacitance vessels capable of providing placental perfusion adequate to sustain the growing fetus. During the process of vascular invasion, the cytotrophoblasts differentiate from an epithelial phenotype to an endothelial phenotype, a process referred to as "pseudovasculogenesis." In pre-eclampsia, cytotrophoblasts fail to adopt an invasive endothelial phenotype. Instead, invasion of the spiral arteries is shallow and they remain small calibered resistance vessels. This may result in the placental ischemia.

Predisposing Maternal Constitutional Factors

Several maternal constitutional factors increase the risk of pre-eclampsia as diabetes mellitus, hypertension, obesity and hyperlipidemia. These conditions may cause oxidative stress and endothelial dysfunction before pregnancy, increasing maternal susceptibility to pre-eclampsia. [51]

Oxidative Stress

Oxidative stress can be defined as an imbalance between reactive oxygen species (ROS) such as superoxide anion radical, hydroxyl radical, nitric oxide and hydrogen peroxide and antioxidants such as vitamin E and C, favoring an overabundance of ROS. [52-53]

Oxidative stress has been proposed to cause endothelial dysfunction, and antioxidants were found to be decreased in pre-eclampsia. [54]

Pre-eclamptic lesions of decidual vessels resemble atherosclerotic lesions, both showing fibrinoid necrosis of the vessel wall and accumulation of lipid-laden foam cells as a hallmark of oxidized low density lipoproteins (oxLDL).[55-56]

Oxidative stress in pre-eclampsia may be caused by abnormal placental development and predisposing maternal constitutional factors. It is therefore unclear whether oxidative stress is a stage in the disease process or a distinct cause of pre-eclampsia. [57]

Immune Maladaptation

Immune maladaptation in the form of maternal immune reaction to paternal antigens in the placenta may cause shallow invasion of spiral arteries and endothelial cell dysfunction mediated by an increased decidual release of cytokines, proteolytic enzymes and free radical species.[58-59]

The interaction between decidual leukocytes and invading cytotrophoblast cells is essential for normal trophoblast invasion and development. [60] Regulatory T cells have been identified as a principle regulator of tolerance during pregnancy. [61] Lower proportion of T-helper cells and deposition of immunoglobulin-M (IgM), complement (C3), and fibrin have been noted in the walls of pre-eclamptic spiral arteries. [62]

Genetic Susceptibility

Susceptibility to pre-eclampsia is highly heritable. Population studies have shown a strong familial susceptibility to pre-eclampsia. [63-64]

Inheritance of pre-eclampsia is probably mediated by multiple genes that increase maternal susceptibility to conditions implicated in the etiology of pre-eclampsia (abnormal placental development, predisposing maternal constitutional factors, oxidative stress and immune maladaptation). [65] However a genetic conflict theory suggested that paternal genes increase nutrient transfer to the fetus and maternal genes limit it. [66-67]