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# Role of Antenatal Fetal Doppler in Correlation with Histopathological, Electron Microscopic and Immuno-Histochemical Findings of Placentas in Prediction of Adverse Perinatal Outcome in Fetal Growth Restricted Pregnancies

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## Abstract

**Objective:** To identify the correlation between antenatal fetal Doppler, histopathological findings of placenta in growth restricted fetuses and the clinical outcome of pregnancy.

**Patient and Methods:** The study group included thirty-one pregnant women with fetal growth restriction of non-anomalous fetus. Ten control cases of uneventful pregnancies which resulted in appropriately grown fetuses for gestational age. Diagnosis of fetal growth restriction was made on clinical and ultrasound basis. Follow up of uterine and umbilical artery Doppler were recorded for all cases. Placental bed biopsies were obtained during caesarean section. All participants were delivered by caesarean section on clinical grounds. All placentas were weighed and tissue blocks were obtained and processed for light, electron microscope examination and immunohistochemistry for evaluating the micro vessel density using CD34 monoclonal antibody marker. Neonatal outcome measures in terms of birth weight, gestational age at delivery, Apgar score, and need for admission to neonatal intensive care unit were reported.

**Results:** Histopathologic examination of growth restriction group showed inadequate trophoblastic invasion of the spiral arterioles in the decidua. The villous capillary density was significantly reduced versus controls (mean 3.2 ± 0.56 per villous and 7.6 ± 0.24 per villous, respectively). Immunohistochemistry study of the villi and decidua showing significant decreased CD34 reactivity that was associated with significant reduction in microvessel density of spiral arteries versus control ($p > 0.001$). Villous vascular abnormalities were positively correlated with abnormal umbilical artery Doppler velocimetry. Whereas decidual vessel abnormalities were significantly correlated with abnormal uterine artery dopplers. Placental pathology was exaggerated in growth restricted fetuses when both umbilical and uterine artery dopplers are abnormal with high incidence of adverse perinatal outcomes in terms of lower gestational age at delivery, birth weight, Apgar Score and 100% admission to neonatal care units.

**Conclusion:** Our findings help to understand the histopathological basis of abnormal umbilical and uterine arteries velocimetry waveforms and adverse perinatal outcome in cases of fetal growth restriction. The most severe adverse clinical pregnancy and perinatal outcome were present when both uterine and umbilical districts were altered.

***Keywords:*** *Fetal Doppler; Histopathology of the Placenta; Intrauterine Growth Restriction*

## Abbreviations

FGR: Fetal Growth Restriction; CS: Caesarean Section; EM: Electron Microscopic; NICU: Neonatal Intensive Care Unit; PE: Pre-Eclampsia; GA: Gestational Age; US: Ultra-Sonographic Examination; RI: Resistance Index; (S/D) ratio: Systolic/End-Diastolic; PI: Pulsatility Index; EM: Electron Microscopic; AGA: Appropriate for Gestational Age

## Introduction

Fetal growth restriction (FGR) complicates a significant proportion of all pregnancies and contributes significantly to increased perinatal morbidity and mortality [1]. The etiology of this condition remains controversial but it is now accepted that (FGR), in a non-anomalous fetus, is associated with fetal hypoxia resulting partially from alterations in the growth and development of the placental villi and their underlying vasculature [2].

The placenta act as an immunological barrier between the mother and the fetus. During the course of pregnancy this barrier becomes extremely thin, less than 2 *μm at term which is only slightly greater than the pulmonary alveolar blood/air barrier* [3]. It is clear that any damage to this barrier from various ischemic risk factors (metabolic, hormonal, genetic and immunological) may be responsible for lesions of the syncytiotrophoblast and villous vessels endothelial cells [4].

The conversion of spiral arteries into utero-placental arteries plays a basic role in the establishment of the physiological placental blood supply. Consequently, the reduced utero-placental blood flow due to inadequate trophoblastic infiltration of the placental vascular bed can be the cause of a variety of pregnancy complications.

Changes in placental functions may be associated with histopathological manifestations in two distinct sites; the placenta itself and the placental bed. Trans placental gaseous transport is dependent upon normal utero-placental and feto-placental blood flow; it therefore follows that a defect in either can impair oxygen delivery to the fetus, rendering him vulnerable to chronic hypoxia which in turn disturbs fetal growth and wellbeing [5].

Doppler ultrasound enables the assessment of blood flow parameters for the adequate and reduced perfusion *in vivo*. Resistance to blood flow in the uterine arteries falls with advancing gestation due to trophoblastic invasion of the uterine spiral arteries. Impaired trophoblastic invasion of the maternal spiral arteries is shown to be associated with high-resistance flow velocity waveforms. Bilateral high-resistance flow velocity waveforms with early diastolic notches at 22 - 24 weeks of gestation are associated with subsequent fetal death, (FGR) and pre-eclampsia (PE) [6].

Umbilical artery Doppler velocimetry reflects function of the placental tertiary villous tree. In pregnancies with FGR fetuses, abnormal umbilical artery velocity waveforms are associated with adverse perinatal outcome [7]. Loss of end diastolic frequencies occurs only when over 75% of placental vascular bed has been obliterated. Loss of end diastolic frequencies is associated with an 85% chance that the fetus will be hypoxemic and a 50% chance that it will also be academic. Growth restricted fetuses with reversed end diastolic frequencies have a ten-fold increase in the perinatal mortality, should be considered as a preterminal condition, few, if any, fetuses will survive without delivery [8].

In this study, we use CD34 immunohistochemical staining as a modality to demonstrate the capillary vascular density in the chorionic villi and deciduas in (FGR) pregnancies.

## Patients and Methods

### Clinical methods

Our participants were recruited among women who attended the Obstetrics department at Al Zahraa university hospital, Cairo, Egypt, for antenatal and intra-partum care and delivered by caesarean section (CS) during the period in between December 2016 till December 2017. Informed consent was obtained from all cases. The study group included 31 women with intrauterine growth restricted fetuses (FGR). Control cases 10 were selected from uneventful pregnancies who delivered of an appropriately growth fetuses for gestational age. All the pregnancies with known vascular maternal disease as chronic hypertension, autoimmune diseases and diabetes, as well, fetuses with known chromosomal or structural abnormalities were excluded from the study. Cases of multiple pregnancies were also excluded.

(CS) was performed on clinical grounds. The indications for (CS) of the controls, which were tried to be at equivalent gestational ages with the study group, were past history of any abnormalities or previous (CS) These indications were chosen on purpose to exclude some other pathology which we could not control. In each case, gestational age was based on precisely dated last menstrual period and was confirmed by the early Ultra-sonographic examination (US).

Diagnosis of FGR was determined when estimated fetal weight less than $10^{th}$ percentile for gestational age (GA) in ultra-sonographic examination and/or birth weight less than $10^{th}$ percentile for (GA).

### Doppler ultrasound examination

Doppler studies were performed using General Electric machine, LOGIC 9. Trans-abdominal color flow/pulsed Doppler examination of both uterine arteries and the umbilical artery were done with 3.5 MHz transducer, color flow mapping, and 50-Hz high pass filter.

All measurements were performed with the mother in a semi- recumbent position. Color flow imaging was used to visualize the ascending branch of the uterine artery. Pulsed Doppler velocimetry was performed with a sample volume of 5 mm. A minimum of three separate recordings of resistance index (RI) was taken for each examination.

The wave contour of the uterine arteries was studied for the presence of a diastolic notch from which the systolic/end-diastolic (S/D) ratio was calculated. Abnormal uterine velocimetry was defined as an average of (left and right) (S/D) ratio > 2.6 and by the bilateral presence of diastolic notching. Umbilical artery waveform was measured from free-floating loop of cord during fetal quiescence. The pulsatility index (PI) was calculated out of the average of three measurements. An abnormal umbilical artery PI was defined as > 2 standard deviations above the mean for gestational age based reference standards [8,9].

Follow up Doppler studies of uterine and umbilical arteries were performed for all cases and the last Doppler record was within the last week before delivery.

### Histopathology, electron microscopic and immunohistochemistry methods

In our attempt to define more clearly the placental histopathological basis of abnormal umbilical and uterine arteries Doppler velocimetry and associated adverse perinatal outcome in (FGR) pregnancies; we have this light and electron microscopic studies of the placental bed and placental villous and vascular structure. The monoclonal antibody against CD34 antigen in human endothelial cell membrane and haemopoietic progenitor cells proved to be a useful marker of villous marker endothelial cells in normal physiologic pregnancy and complicated pregnancy [10].

All placentas were weighted and two full thickness blocks of placental tissue were obtained from the area of the cord insertion and processed for light, electron microscopic (EM) examination and immunohistochemistry for evaluating the micro vessel density using CD34 monoclonal antibody marker. The monoclonal antibody against CD34 antigen in human endothelial cell membrane and haemopoietic progenitor cells proved to be a useful marker of villous marker endothelial cells in normal physiologic pregnancy and complicated pregnancy [11,12].

In this study, we use CD34 immunohistochemical staining as a modality to demonstrate the capillary vascular density in the chorionic villi and deciduas in (FGR) pregnancies. Neonatal outcome measures in terms of birth weight, gestational age at delivery, Apgar score and need for admission to neonatal intensive care unit (NICU) were reported.

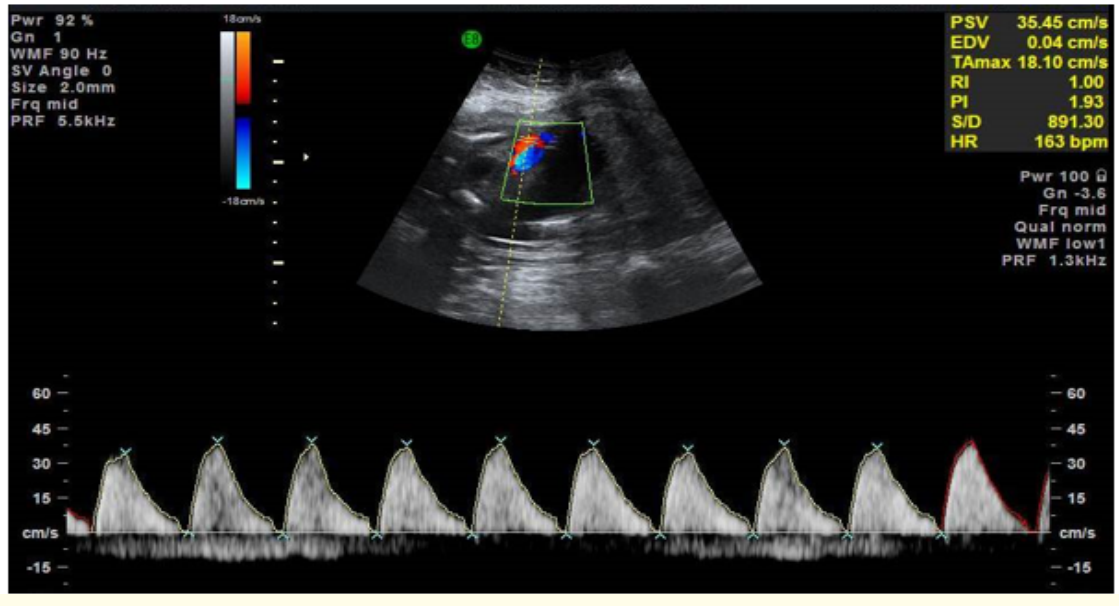


***Figure 1:*** *Color Doppler umbilical artery wave form demonstrating absent end diastolic frequency. This is less severe by comparison with figure 2.*

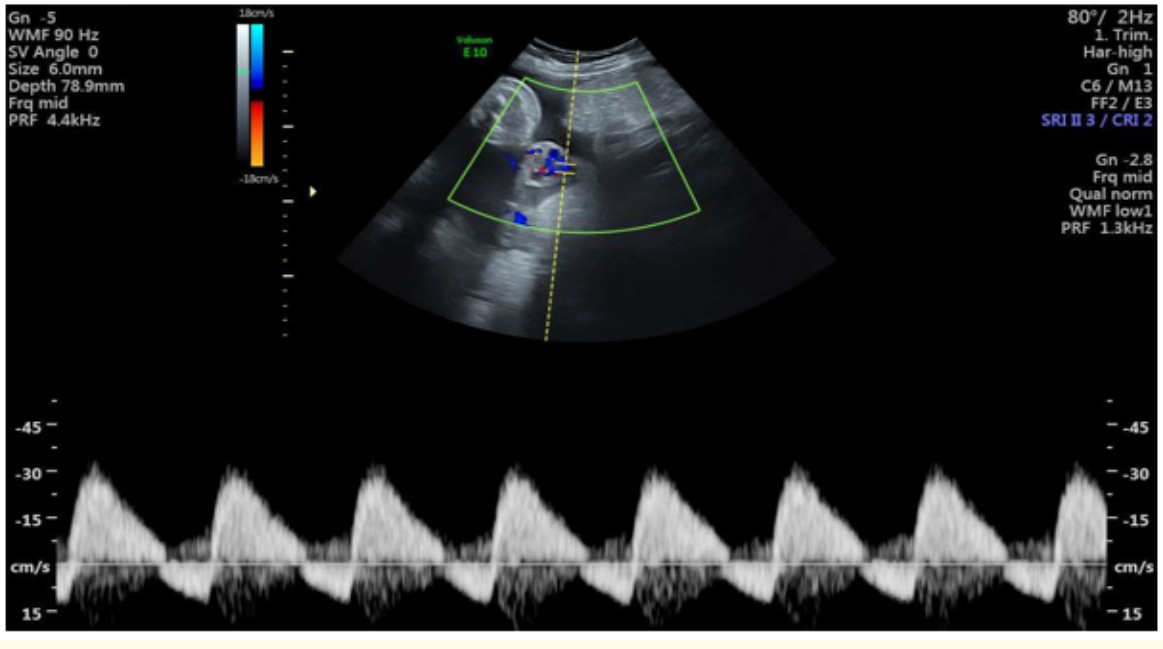


***Figure 2:*** *Color Doppler umbilical artery wave form demonstrating reversed end diastolic frequency.*

### Statistical analysis

Statistical presentation and analysis of the present study was conducted, using the mean, standard error, Student t-test [Unpaired], paired t-test, analysis of variance [ANOVA] test, Linear Correlation Coefficient [r] and chi-square by SPSS V. 13.

## Results

### Clinical Findings

When the maternal age and parity compared between the AGA (appropriate for gestational age) (n = 10) and the (FGR) (fetal growth restriction) pregnancies (n = 31), there was no significant correlation to the fetal growth restriction with P Value (P < 0.05). The mean maternal age of AGA and FGR was (28.1 ± 8.1) and (29.4 ± 8.3) respectively. The mean Parity of (AGA) and (FGR) was (2.63 ± 1.98) and (3.7 ± 3.6) respectively (Table 1).

| Variables | AGA n = 10 | FGR n = 31 | P Value |
|---|---|---|---|
| Maternal age (years) | 28.1 ± 8.1 | 29.4 ± 8.3 | P < 0.05 |
| Parity | 2.63 ± 1.98 | 3.7 ± 3.6 | P < 0.05 |

***Table 1****: Maternal age and parity of AGA (appropriate for gestational age) and FGR (fetal growth restriction) pregnancies.*

The fetal outcome was compared between the AGA and the FGR pregnancies, demonstrate significant reduction of gestational age at birth, birth weight, placental weight and Apgar score with P Value (P < 0.001). The mean Gestational age at birth by weeks of AGA and FGR was 36.4 ± 3.2 and 30.2 ± 2.3 respectively. The mean Birth weight by grams of AGA and FGR was 2810 ± 426.42 and 1690 ± 452.95 respectively. The mean of placental weight by grams of AGA and FGR was 453.83 ± 81.58 and 302.63 ± 90 respectively. Apgar score mean of AGA and FGR was 8.23 ± 1.43 and 5.88 ± 1.39 respectively (Table 2).

There was significant increase in the need of admission in of the (FGR) neonates into the (NICU) P Value (P < 0.001) as the percentage of AGA neonates who needed (NICU) admission was 11.9% while of (FGR) neonates was 52.5% (Table 2).

| Variables | AGA n = 10 | FGR n = 31 | P Value |
|---|---|---|---|
| Gestational age at birth (weeks) | 36.4 ± 3.2 | 30.2 ± 2.3 | P < 0.001 |
| Birth weight (grams) | 2810 ± 426.42 | 1690 ± 452.95 | P < 0.001 |
| Placental weight (grams) | 453.83 ± 81.58 | 302.63 ± 90 | P < 0.001 |
| Associated preeclampsia | - | 16/31 (51.61%) | |
| Apgar score | 8.23 ± 1.43 | 5.88 ± 1.39 | P < 0.01 |
| NICU (%) | 11.9 | 52.5 % | P < 0.001 |

***Table 2:*** *Fetal outcome of AGA and FGA groups.*

So, the fetal outcome was worse in the (AGA) neonates as compared to the (FGR) neonates.

The uterine Doppler velocimetry and umbilical Doppler velocimetry were significantly abnormal in FGR fetuses as compared to AGA fetuses with P Value (P < 0.001). 65% of FGR fetuses had abnormal Uterine Doppler velocimetry and only (10%) of AGA fetuses. 61% of FGR fetuses had abnormal Umbilical Doppler velocimetry and only (10%) of AGA fetuses (Table 3).

| Variables | AGA n = 10 | FGR n = 31 | P Value |
|---|---|---|---|
| Abnormal uterine Doppler velocimetry (N%) | 1 | 20 | P < 0.001 |
| Abnormal umbilical Doppler velocimetry (N %) | 1 | 19 | |

***Table 3**: Abnormal Uterine and Umbilical Doppler velocimetry in AGA and FGA groups.*

## Histopathology

**Control group:** The control specimens showed the typical well-known normal structure of human placental fetal blood vessels. The diffusion barrier between maternal and fetal circulations comprised 3 layers, (trophoblast, trophoblast basement membrane and endothelium). In many regions of normal placentas fetal capillaries were so close to the trophoblast that their basement membranes fused, reducing the diffusion barrier and forming the so called Vasculosyncytial membrane. The mean capillary density was 2.6 + 0.36 per villous.

Examination of the decidua displayed many foci of trophoblastic invasion of the spiral arterioles (Figure 3).

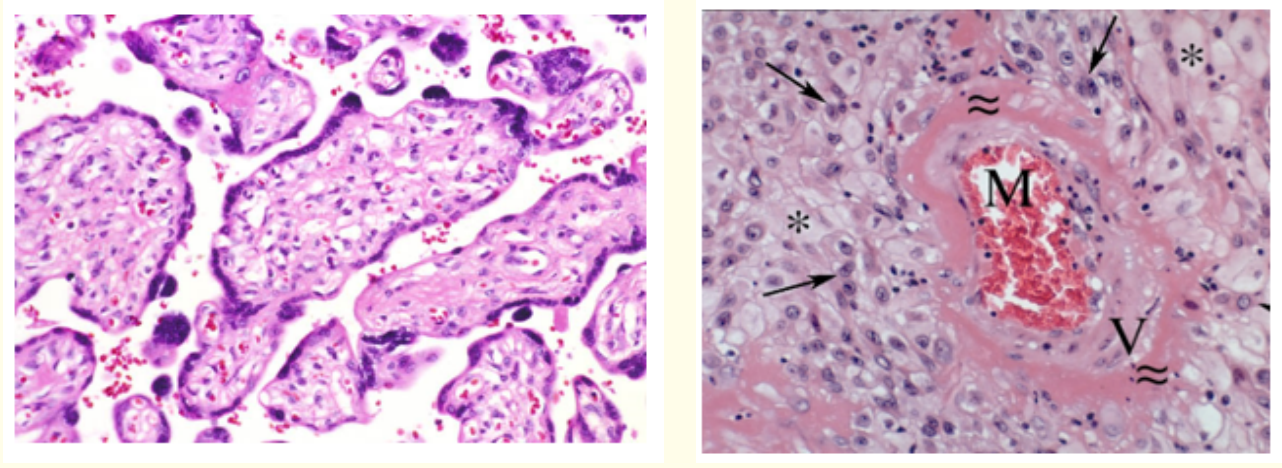


***Figure 3:** Control placenta: a) Section showing chorionic villi with normal pattern, frequent fetal vessels, contained fetal red cells (H&Ex400).*

*b) Section in the decidua showing trophoblastic invasion of spiral arteriole. Uterine spiral artery (V) containing maternal blood (M). Decidualized stroma contained invasive trophoblasts (arrows) which have begun to modify the vessel wall (H&Ex400).*

**FGR group:** Villi demonstrated frequent knobs, micro calcifications, sclerosis and fibrinoid degeneration. The mean capillary density of the villi was significantly decreased (1.3 + 0.58 per villous) and many were devoid of capillaries. Examination of the decidua revealed wide areas of infarction with hyalinosis and fibrosis. There was thickened vascular spaces and inadequate trophoblastic invasion (Figure 4).

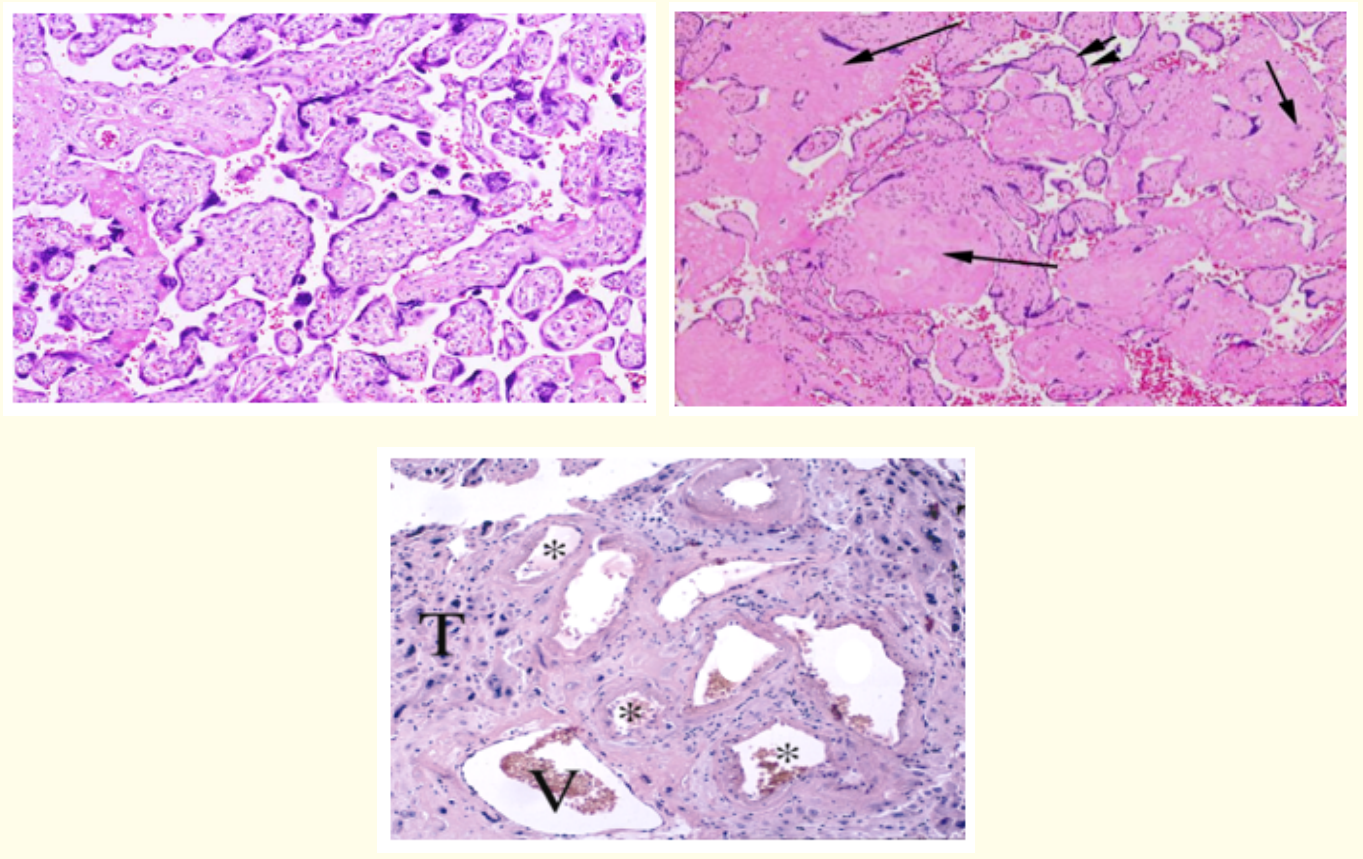


***Figure 4:*** *Placenta of FGR: a) Section in of Chorionic villi showing early ischemic features (hyalinization and fibrinoid change). The capillary density was ranged from 2 up to 3 villous(H&Ex100).*

*b) Section showed advanced ischemic features with necrosis and hyalinized villi. Capillaries were reduced or absent (H&Ex100).*

*c) Section in the decidua showing Failure of invasive trophoblasts (T) to penetrate the maternal spiral arteries (*) a case of FGR. A maternal vein (V) was noticed (H&Ex200).*

## Electron microscopy

**Control group:** The endothelial cells of a fetal capillary from placentas of healthy women is formed of flattened endothelial cells with centrally locating nucleus resting on a basement membrane and supported by delicate collagenous tissue. Larger vessels were lined with extremely flattened endothelium and the lumen may acquire two or more blood cells (Figure 5).

**FGR Group:** Examination under transmission electron microscope demonstrated narrowing of vascular lumen to accommodate one blood cell. There was plumbed endothelial cell with luminal localization of the nucleus to be pushed into the lumen. The endothelial cell showed multiple processes connected to the underlying basement membrane and proliferated smooth muscle cells. The basement membrane was thickened and rich in collagen fibers. There was multiple Vasculosyncytial membrane breakage (Figure 5).

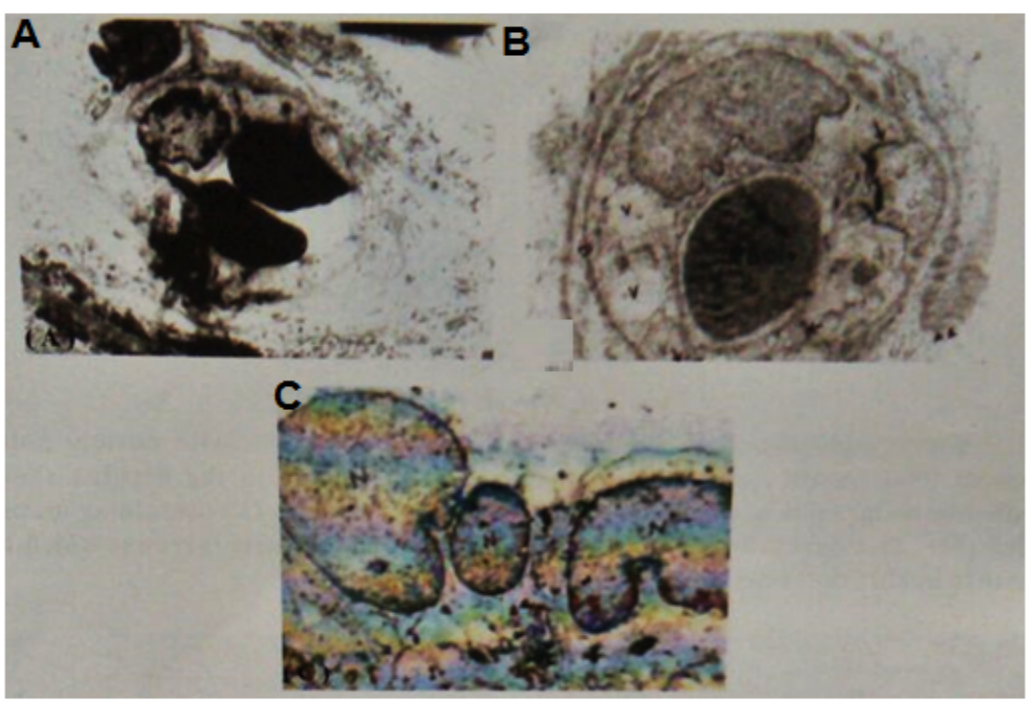


***Figure 5 :*** *An electron micrograph of a placenta in:*

*A) In a control group showing fetal capillary formed of flattened endothelium and contained two RBC in its lumen (x400).*

*B) In FGR group showing fetal capillary with narrowing of the lumen that contain only one RBC, the lining endothelial cells contain abundant cytoplasm, vacuoles (V), widening in interendothelial junctions and thickening of capillary basement membrane (x 400).*

*C) Showing a fetal stem vessel in FGR placenta. The nuclei of endothelial cells are deeply indented and situated close to the lumen (N), notice the wide interendothelial gaps (x600).*

## Immunohistochemistry

Immunoreactivity of the CD34 was demonstrated to mark the mature endothelial cells lined capillaries and the newly formed micro vessel before lumen formation. In control group, positive reactivity of CD34 monoclonal antibody was numerous per villous (mean 8.6 + 0.65). The mean decidual micro vessel density was 10.95 + 0.21 (Table 4).

| Micro vessel density | AGA (control group) n = 10 | FGR (study group) | | P Value |
|---|---|---|---|---|
| | | Uterine artery Doppler | | |
| | | Normal n = 11 | Abnormal n = 20 | |
| Villous | 8.6 + 0.65 | 5.1 ± 0.22 | 3.02 + 0.45 | P < 0.001 |
| Decidual | 10.95 + 0.21 | 7.7 ± 0.13 | 4.65 + 0.98 | P < 0.001 |

***Table 4:*** *Micro vessel density in the villi and the decidua, in control AGA (appropriate for gestational age) and FGR (fetal growth restricted) pregnancies.*

In FGR group the mean micro vessel density per villous was significantly decreased or may be absent (mean = 3.02 + 0.45), and the decidual micro vessel density was 4.65 + 0.98 (Table 4).

From the FGR group which is total 31, Umbilical Doppler flow was found to be normal in 12 (38%) and abnormal in 19 (61%).

Placental histopathology in (FGR) pregnancies with normal and abnormal Umbilical artery velocimetry demonstrate, Reduced capillary density in (33%) and (84%) with normal and abnormal Umbilical Doppler flow respectively. Breakdown of vasculo-syncytial membrane in (25%) and (52%) with normal and abnormal Umbilical Doppler flow respectively. Micro-infarction in (41%) and (57%) with normal and abnormal Umbilical Doppler flow respectively, so the placental pathological changes were more severe in FGR group with abnormal Umbilical Doppler flow (Table 5).

| Histopathologic variable | Control (AGA) | FGR | | P Value |
|---|---|---|---|---|
| | | Umbilical Doppler flow | | |
| | | Normal N = 12 | Abnormal N = 19 | |
| Reduced capillary density | - | 4 | 16 | P < 0.001 |
| Breakdown of vasculo-syncytial membrane | - | 3 | 10 | P < 0.001 |
| Micro-infarction | 1 | 5 | 11 | P < 0.01 |

***Table 5:*** *Frequencies of placental histopathologic features in AGA (appropriate for gestational age) and FGR (fetal growth restricted) pregnancies with normal and abnormal umbilical artery velocimetry.*

Placental bed biopsy histopathology in (FGR) pregnancies with normal and abnormal Uterine artery Doppler velocimetry demonstrate, Inadequate trophoblastic invasion in (18%) and (80%) with normal and abnormal Umbilical Doppler flow respectively. Thrombosis or luminal obliteration of spiral arteries in (9%) and (60%) with normal and abnormal Umbilical Doppler flow respectively, so the placental bed pathological changes were more severe in FGR group with abnormal Uterine Doppler flow (Table 6).

| Histopathologic variable | AGA N = 10 | FGR | | P Value |
|---|---|---|---|---|
| | | Uterine artery Doppler flow | | |
| | | Normal N = 11 | Abnormal N = 20 | |
| Inadequate trophoblastic invasion | - | 2 | 16 | P < 0.001 |
| Thrombosis or luminal obliteration of spiral arteries | - | 1 | 12 | P < 0.01 |

***Table 6:*** *Frequencies of histopathologic features of placental bed biopsy in AGA (appropriate for gestational age) and FGR (fetal growth restricted) cases with normal and abnormal uterine artery Doppler velocimetry.*

Placental and placental bed biopsy (decidual) histopathology was compared when both Umbilical artery pulsatility index (PI) and Uterine artery resistance index (RI) were both normal and both abnormal, demonstrate abnormal histopathology in 25% when both Umbilical artery (PI) and Uterine artery (RI) were normal and 70 - 90% when both were abnormal, so the effect was more severe when both were abnormal (Table 7).

| | Uterine artery resistance index (RI) | | | |
|---|---|---|---|---|
| | Normal | | Abnormal | |
| Umbilical artery pulsatility index (PI) | Normal | Abnormal | Normal | Abnormal |
| N (31) | 4 | 4 | 6 | 17 |
| Pathologic placenta | 1 | 4 | 3 | 16 |
| Pathologic placental bed biopsy (decidual) | 1 | 1 | 4 | 12 |

***Table 7:*** *Histopathologic features of the placentas in relation to uterine and umbilical artery Doppler velocimetry.*

The fetal outcome of the FGR pregnancies was compared when both Umbilical artery pulsatility index (PI) and Uterine artery resistance index (RI) were both normal and both abnormal, there was reduction of gestational age at birth, birth weight, placental weight and Apgar score. The mean Gestational age at birth by weeks was 35.2 ± 2.3 when both Umbilical artery (PI) and Uterine artery (RI) were normal and 31.8 ± 1.9 when both were abnormal. The mean Birth weight by grams was 2052 ± 460 when both Umbilical artery (PI) and Uterine artery (RI) were normal and 1261 ± 510 when both were abnormal. The mean placental weight by grams was 328 ± 60 when both Umbilical artery (PI) and Uterine artery (RI) were normal and 248 ± 87 when both were abnormal. Apgar score mean was 7.53 ± 1.43 when both Umbilical artery (PI) and Uterine artery (RI) were normal and 4.86 ± 2.61 when both were abnormal (Table 8).

The need of admission of the FGR neonates to the neonatal intensive care unit (NICU) was increase when both Umbilical artery pulsatility index (PI) and Uterine artery resistance index (RI) were both abnormal to (100%) and only (10%) when both indices were normal (Table 8).

| | Uterine artery resistance index (RI) | | | |
|---|---|---|---|---|
| | Normal | | Abnormal | |
| Umbilical artery pulsatility index (PI) | Normal | Abnormal | Normal | Abnormal |
| N (31) | 4 | 4 | 6 | 17 |
| Gestational age (by weeks) at delivery | 35.2 ± 2.3 | 35.4 ± 3.9 | 36.1 ± 2.7 | 31.8 ± 1.9 |
| Birth weight (gm) | 2052 ± 460 | 1890 ± 581 | 2230 ± 309 | 1261 ± 510 |
| Placental weight (gm) | 328 ± 60 | 325 ± 90 | 331 ± 76 | 248 ± 87 |
| Apgar score | 7.53 ± 1.43 | 6.13 ± 1.52 | 6.33 ± 1.36 | 4.86 ± 2.61 |
| NICU | 2 (10%) | 3 (15%) | 5 (25%) | 20 (100%) |

***Table 8:*** *The fetal outcome of growth restricted pregnancies in relation to uterine and umbilical artery Doppler velocimetry.*

So the fetal outcome was worse in the FGA when with both Umbilical artery pulsatility index (PI) and Uterine artery resistance index (RI) abnormal than when both were normal.

## Discussion

The present study established the ultra-structural changes that occurred in placental vasculature and micro vessel density in placental bed biopsy in cases of FGR. These abnormalities in feto-maternal circulation were shown to be significantly correlated with adverse perinatal outcome. histopathologic examination in cases of FGR is recommended specially, idiopathic FGR, to guard against recurrence. Our findings help to understand pathological basis of abnormal umbilical and uterine arteries Doppler velocimetry and adverse perinatal

outcome in cases of FGR. Our findings were in parallel to those previously described by Ferrazzi., *et al* [13]. Viscardi and Sun [14] reported increased villous infarcts, hypo vascularity, fibrosis, thickening of the basal membrane, obliterative endo-arteritis, cytotrophoblast proliferation and syncytiotrophoblast knotting in placentae of FGR. The presence of two or more of these placental lesions has been shown to be related to perinatal mortality and morbidity [15]. Previous light microscopy studies have concluded that the sectional area of placenta occupied by non-muscularized villi is reduced in cases of FGR as compared with gestational age-matched controls [16].

Another important histopathological manifestation of impaired placental implantation is atherosis. Acute atherosis defined as fibrinoid necrosis of the vessel wall with sub intimal accumulations of lipid-laden cells, has been documented in percent of the maternal spiral arterioles of FGR pregnancies. Thrombosis or luminal obliteration of spiral arteries and increased extra villous trophoblast were also, noticed in placental bed biopsy of FGR pregnancies. Incomplete trophoblastic invasion, reduced micro vessel density, atherosis and thrombosis of implantation site vessels may well result in FGR by causing diminished blood supply to the uteroplacental unit which is clinically expressed as high impedance to flow in uterine arteries with abnormal Doppler flow velocimetry [17].

In this study, EM study of the placenta in cases of FGR revealed a number of pathological changes in the form of endothelial cell damage, placental barrier breakage, smooth muscle proliferation of fetal placental vessels and villous vascular density. This comes in concordance with a number of previous studies. Scanning EM of both villous tissue and vascular casts, have confirmed the hypothesis of peripheral villous maldevelopment in severe preterm FGR [18].

In the present study, EM examination revealed smooth muscle proliferation of placental arteries. Furthermore, smooth muscle formed beds and cytoplasmic protrusions in both arteries and veins. This might explain the attenuated relaxing vascular response to endothelial nitric oxide in FGR [3].

In this study, immunostaining with CD34 demonstrates that the capillary vascular density in the fetal chorionic villi and the deciduas is significantly decreased in cases of FGR and this was in parallel to those previously reported by Egbor., *et al.* [19] and Zygmunt., *et al* [20].

Egbor., *et al.* [19] have used CD 34 immunostaining to do quantitative studies on placental villous and vascular architecture in cases of pre-eclampsia (PE) and FGR by the application of stereological analysis to estimate different parameters such as volume, surface areas, lengths and diameter of intermediate and terminal villi and their fetal vasculature and result that FGR are there than PE was associated with significant reduction in all parameters.

In present research, the prevalence of placental villous and vascular abnormalities were positively correlated with abnormal umbilical artery Doppler velocimetry readings this might explain the previous reports of Karsdrop., *et al.* [21]; Ott [22] and Madazli., *et al.* [23] as abnormal umbilical artery Doppler velocimetry was shown to be associated with adverse prenatal outcome.

In the present study, the endothelium of placental fetal stem vessels in cases of FGR showed long processes and disruption of the cell membrane. Similar to that described by Wang., *et al.* [24] whose suggested that activation and injury of endothelial cells occurred in FGR umbilical cord endothelial cells. Furthermore, the circulating endothelial micro particles directly affect the endothelium and impair its function [25]. Circulating endothelial micro particles were isolated from blood of cases with FGR and not from healthy pregnant women [26].

The response of the endothelium is restricted by the counteractive effect of the surrounding microenvironment, e.g. in the brain, to maintain blood brain barrier and low permeability properties. Endothelial dysfunction or activation contributes to a variety of diseases states [27].

Shear stress is the frictional tangential force imposed on the vessel wall when blood flows through a vessel. Normally, elevations of the placental blood flow and physiologic shear stress may be partly responsible for the increase in placental arterial endothelial nitric oxide production during pregnancy which had a direct vascular relaxing effect. The absence of elastic lamina in placental arteries means that there is little physiologic barrier between endothelial and smooth muscle cells. Therefore, a grater efficacy of endothelial derived vasodilatory agents in placental arteries compared with maternal arteries [28]. However, in FGR in addition to endothelial dysfunction, placental arteries exhibited an attenuated vasodilatory response to nitric oxide [29].

The syndrome of FGR during the latter half of pregnancy is believed to result from impaired placentation in early gestation [30]. Deficient placentation is characterized by inadequate trophoblast invasion into the maternal spiral arteries and a failure to develop low-resistance utero-placental circulation [9]. Failure of the extra villous trophoblast to invade the intramyometrial portion of the spiral arteriole has been described in pregnancies complicated by FGR [31]. In this study, light and EM examination of decidua revealed that in all pregnancies with AGA fetuses, complete trophoblastic invasion of spiral arterioles has been observed, whereas, percentage of pregnancies complicated by FGR failed to show this normal pregnancy adaptation. Immuno-histochemistry obscured the microvascular density of spiral arteries in the decidua of FGR pregnancies which was significantly reduced.

In our research, the frequency of placental vessel abnormalities was significantly correlated with abnormal umbilical artery Doppler velocimetry.

As well, the micro vessel density of spiral arteries in the decidual was significantly reduced in FGR cases associated with abnormal uterine artery Doppler readings.

Decreasing vascularization of placental bed leads to lower nutritional status for the fetus resulting in FGR. These data confirm that abnormal uterine artery Doppler velocimetry well reflects placental bed arteriopathy of pregnancies complicated by FGR. Indeed, uterine artery Doppler studies enables us to obtain blood flow parameters for reduced perfusion on the maternal side of utero-placental circulation. The relationship between uteroplacental vascular pathology and abnormal uterine artery Doppler flow has been demonstrated by previous studies [32,33]. Despite significant differences in micro vessel density of the spiral arteries in the decidua between cases of FGR and AGA uterine artery Doppler frequencies still normal in 11 out of 31 cases of FGR consequently abnormal uterine artery readings in cases with FGR must indicates marked reduction in maternal blood supply to the placenta at some point in pregnancy when termination is a must to protect the fetus from adverse intrauterine environment, simultaneous abnormal umbilical Doppler readings at this specific point in pregnancy will potentiates the decision making up of pregnancy termination if extra uterine survival is possible.

Considering umbilical and uterine artery Doppler velocimetry together, we demonstrated that; villous pathology and abnormal decidua biopsy are best reflected by abnormal umbilical and uterine artery flow velocimetry waveforms, respectively. Therefore, this finding clearly demonstrates that umbilical artery Doppler velocimetry reflects the placental side and uterine Doppler velocimetry reflects the maternal side of fetomaternal circulation. When co-existent abnormal umbilical and uterine artery Doppler readings are present, our results displayed a parallel correlation of pathological ultra-structural changes of placenta and placental bed biopsy to vascular resistance and adverse perinatal outcome in terms of gestational age at delivery birth weight, Apgar score and the NICU.

## Conclusion

Our findings help to understand the histopathological basis of abnormal umbilical and uterine arteries velocimetry waveforms and adverse perinatal outcome. In FGR pregnancies, the most severe adverse clinical pregnancy and perinatal outcome were present when both uterine and umbilical districts were altered. We may speculate that, as the magnitude of abnormal placentation increases, intervillous blood flow decreases leading to placental vascular pathologies and in turn both alter fetal growth and wellbeing.

## Disclosure

The authors declared no conflict of interest.

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