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ORIGINAL ARTICLE



# **Correlation of Doppler Velocimetry of Uterine and Umbilical Arteries with Placental Pathology in Pregnancy Associated with Intrauterine Growth Restriction**

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Abstract To correlate histomorphology of the placenta with Doppler studies of uterine and umbilical arteries. A comparative observational study conducted on 75 pregnant women divided into 2 groups: Group 1 included 25 women with appropriate for gestational age fetuses. Group 2 included 50 women with FGR. Uterine and umbilical artery Doppler, study of placental pathology and immunohistochemistry of placental villous tissues were evaluated. There was a significant difference between the two study groups regarding both abnormal uterine (0 vs. 58%) and umbilical artery (0 vs. 58%) Doppler (p < 0.001). Syncytial knots > 30% (44 vs. 60%), fibrinoid necrosis > 5% (8 vs. 46.7%), placental infarction > 5% (0 vs. 15%), perivillous fibrinoid deposition > 5% (1.8 vs. 16.7%) (p < 0.001) but not calcifications (56 vs. 60%, p = 0.121) were significantly higher in FGR placentas. A statistically significant (p < 0.001) increase in the expression of VEGF in FGR placentas when compared with normal placentas. Abnormal uterine artery but not umbilical artery Doppler was significantly associated with abnormal placental pathology. Women with both abnormal uterine and umbilical artery

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<sup>1</sup> Obstetrics and Gynecology Department, Cairo University, 481 King Faisal Street, Haram, Giza 12111, Egypt Doppler velocimetries were delivered earlier and their babies had lower mean birth and placental weight (p < 0.001). Incidence of abnormal placental pathology was significantly higher in this specific group of FGR pregnancies (p < 0.001). There is high association between abnormal uterine and umbilical artery Doppler and placental pathology in FGR associated pregnancies.

Trial Registration NCT03081754.

**Keywords** Uterine artery Doppler · Umbilical artery Doppler · FGR · Placental pathology

# Introduction

Battaglia and Lubchenco classified small-for-gestational age (SGA) neonates as those whose weights were below the 10th percentile for their gestational age. Such infants were shown to be at increased risk for neonatal death [1].

As many as 25–60% of SGA infants are thought to be appropriately grown when maternal ethnic group, parity, weight, and height are considered [2]. These SGA infants remain significantly smaller during surveillance to 2 years compared with appropriate-for-gestational age neonates, but they do not show differences in measures of metabolic risk [3].

Individual or customized fetal-growth potential has been proposed in place of a population-based cutoff [4]. Such optimal projections are based on maternal race or ethnicity. Fetal-growth restriction was included by Brosens et al. [5] as one of the "great obstetrical syndromes" associated with defects in early placentation.

Notably, several immunological abnormalities have been associated with fetal-growth restriction. This raises the prospect of maternal rejection of the "paternal

Materials and Methods

A comparative observational study was conducted on 75 pregnant women who attended Kasr Alainy Maternity Hospital, Faculty of Medicine, Cairo University during the period from March 2014 to December 2016. Informed written consents were obtained from all participants after obtaining of local ethical committee approval.

The 75 women included were divided into 2 groups: Group 1 is the control group included 25 uneventful pregnancies with appropriate for gestational age fetuses. Caesarean sections were performed on clinical grounds for abnormal fetal presentation or prior cesarean delivery. Group 2 included 50 women with FGR defined as abdominal circumference > 2SD below the mean for gestational age and also confirmed by the serial assessment of the fetal growth parameters [18]. Gestational age was based on the precisely dated last menstrual period and ultrasonographic examination of crown-rump length in the first trimester.

Pregnancies with known vascular maternal disease (chronic hypertension, autoimmune diseases, and diabetes) and fetuses with structural abnormalities, twins were excluded from the study.

Clinical data were collected during subsequent antenatal visits and at the discharge from hospital. After first trimester enrolment, antenatal visits were scheduled at 4 weeks intervals. After the initial sonogram obtained at 11–14 weeks of pregnancy, further ultrasonography evaluations were scheduled at 18–22 and 28–32 weeks of pregnancy. When indicated, a conservative management plan of FGR was undertaken according to a defined protocol including antenatal visits, ultrasound surveillance. The frequency of fetal surveillance was assessed at each visit according to the maternal and fetal conditions.

Doppler studies were performed within the last week before delivery using (Voluson 58-GE ultrasound Seoul, South Korea) machine equipped with a 3.5-MHz transducer, color-flow mapping, and a 50-Hz high-pass filter; all measurements were performed with the mothers in a semi recumbent position. Color-flow imaging was used to visualize the ascending branch of the uterine arteries. Pulsed Doppler velocimetry was performed with a sample volume of 5 mm. A minimum of three separate recordings 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 and by the bilateral presence of diastolic notching (Fig. 1). Umbilical artery waveform was measured from free-floating loop of cord during fetal quiescence. The pulsatility index (PI)

semiallograft." Rudzinski and colleagues studied C4d, a component of complement that is associated with humoral rejection of transplanted tissues. They found this to be highly associated with chronic villitis—88% of cases versus only 5% of controls—and with reduced placental weight [6]. Greer and associates studied 10,204 placentas and reported that chronic villitis was associated with placental hypoperfusion, fetal acidemia, and fetal-growth restriction and its sequelae [7]. Kovo et al. [8] found that chronic villitis is more strongly associated with fetal-growth restriction than with preeclampsia. Redline described the findings of activated maternal lymphocytes among fetal trophoblast [9].

The introduction of color Doppler technology has provided the first opportunity for repetitive noninvasive hemodynamic monitoring in pregnancy. There is ample evidence that Doppler indices from the fetal circulation can reliably predict adverse perinatal outcome in SGA pregnancy [10].

Evaluation of the placenta is extremely important in attempting to understand the pathophysiology of FGR. Only with careful gross and histologic evaluation, along with clinical pathologic correlation, can the underlying causes and recurrence risks be understood. There are many well-established causes of FGR but in idiopathic FGR, there is no obvious fetal or maternal cause. The placentas of these 'idiopathic' FGR babies might hold the key to the etiology of the growth restriction. The contribution of placental changes remains controversial [11].

Angiogenesis is a placental factor playing an important role in the development of FGR [12]. Angiogenesis plays a role in the development of the villous vasculature and the formation of terminal villi in the human placenta. FGR occurs as a result of the failure of elongation, branching, and dilation of the capillary loops and of terminal villous formation [13].

Angiogenesis can be identified in tissue by the use of CD34 monoclonal antibody and estimation of microvessel density counting (MVD). Vascular endothelial growth factor (VEGF) has been identified as positive regulators of angiogenesis [14]. It plays an essential role in the formation of new blood vessels. In pregnancy, VEGF participates in the proliferation, migration, and metabolic activity of trophoblasts [15]. It is expressed by human villous and extravillous trophoblasts, and acts as a modulator of tissue differentiation and placental angiogenesis [16].

Researches are trying to find predictive parameters for FGR, with the goal of reaching an early diagnosis, which would lead to a better management of the condition [17].

The aim of our trial is to study the correlation of histomorphology of the placenta and placental bed with Doppler velocimetries of the uterine and umbilical arteries in normal and FGR pregnancies.



Fig. 1 Uterine artery Doppler in FGR with abnormal resistance

(maximum velocity – minimum velocity/mean velocity) was calculated and the average of three measurements was used. An abnormal umbilical artery PI was defined as standard deviations above the mean for gestational age based reference standards [18].

The results of umbilical artery (UA) Doppler velocimetry were categorized as normal (end-diastolic velocity < 90th percentile of our reference curve), increased (end-diastolic velocity  $\ge$  90th percentile), absent, and reversed [19]. Patients were admitted for close surveillance in the case of worsening of maternal or fetal conditions (e.g. absent or reversed UA blood flow, and severe preeclampsia) [20].

#### **Tissue Samples**

The general shapes of placentas were assessed (Fig. 2). The collected placentas were weighed by trimming the membranes and umbilical cord. Then the diameters and thickness of placentas were noted. The position of insertion of umbilical cord on the fetal surface of placenta was observed. Transverse cuts were made through the maternal surface at a distance of 1–2 cm in bread loaf manner and examined for the pale areas. All placentas were immersed in 10% formalin overnight and examined on the next day. For each placenta, blocks containing cord, membrane and full thickness of villous tissue were prepared. Whole thickness villous tissue blocks were obtained from three zones named central, peripheral and intermediate zones to include all areas of placenta.

Placental bed biopsies were obtained at caesarean sections with direct visualization of the placental site. Biopsies of at least 1 cm were taken. The specimens were fixed in buffered formalin. The tissues were processed and stained with haematoxlyin and eosin. Microscopic study of placenta was carried out utilizing a set of standard criteria for villous and intervillous lesions [21]. For studying these criteria, 8 random microscopic fields were chosen and 100 villi were counted in each field and studied for the presence of syncytial knots > 30% in one field, fibrinoid necrosis > 5% in one field and placental infarction > 5% in one field. The intervillous space was evaluated for chorangiosis, perivillous fibrinoid deposition > 5% in one field, infarctions, calcification and thickened hyalinosed blood vessels (Fig. 3). For statistical purpose such changes are labeled as "Abnormal placenta".

#### Immunohistochemistry

Expression of VEGF and CD34 was analyzed in placental villous tissues. Samples  $(1.5 \times 1.5 \times 1 \text{ cm} \text{ in diameter})$  taken from the maternal surface of each placenta; infarct areas were excluded from the study. All tissues were fixed in formalin, embedded in paraffin, and cut into 5-µm-thick sections, which were collected on slides coated with poly-L-lysine. After the paraffin was removed, the sections were rehydrated.

Immunostaining was performed by the streptavidin–biotin–peroxidase method. Endogenous peroxidase activity was blocked using 3% hydrogen peroxide. Antigen retrieval was carried out in a microwave oven for 15 min in 10 nM citrate buffer (pH 6.0) for VEGF. The sections were incubated at room temperature for one hour with EP1176Y rabbit polyclonal antibodies reactive with VEGF (1:100; Genova, Spain), CD34 mouse monoclonal antibodies Ventana. USA). After washing in phosphate-buffered saline with Tween-20, the tissues were incubated with a biotin-conjugated secondary antibody and then with a biotin-streptavidin complex for 30 min at room temperature. Reactions were visualized with 3, 3-diaminobenzidine tetrahydrochloride (DAB). Sections were counterstained with hematoxylin, rinsed, and mounted.

Evaluation of immunohistochemical staining of CD34 was done using microvessel density (MVD) Determination by counting the number of microvessels per unit area of tissue section [22].

Tissue sections were initially screened microscopically at low power  $(100\times)$  to identify the areas of highest vascularization ("hot spots"). Five high-power  $(400\times)$  fields were then chosen randomly, and the number of microvessels in each high-power field  $(0.09 \text{ mm}^2)$  was counted for each sample with the use of an ocular grid. MVD for each sample was taken as the mean of the five values obtained [23].



Fig. 2 FGR placenta. a Fetal surface, b maternal surface, c hemorrhagic infarctions, d infarctions and calcifications

To evaluate the immunohistochemical staining of VEGF, the intensity and localization of the staining reaction in chorionic villous stromal cells, vascular smooth muscle cells, villous vascular endothelial cells, cytotrophoblasts, syncytiotrophoblasts, and extravillous trophoblasts was done (Fig. 4). Immunoreactivity for antibodies was scored using a semi-quantitative scale for intensity of staining: 0 negative, no staining; 1+ weak positive; 2+ moderately positive; 3+ strongly positive [16].

Data were statistically described in terms of mean  $\pm$  standard deviation ( $\pm$  SD), median and range, or frequencies (number of cases) and percentages when appropriate. Student *t* test, Mann–Whitney *U* test, Chi-square ( $\chi^2$ ) test were used accordingly. Multivariate logistic regression analysis was used to test for the preferential effect of abnormal Doppler parameters on the presence of placental pathology. Accuracy was represented using the terms sensitivity, specificity, +ve predictive value, –ve predictive value, and overall accuracy. *p* values less than 0.05 was considered statistically significant. All statistical calculations were done using computer program SPSS (Statistical Package for the Social Science; SPSS)

Inc., Chicago, IL, USA) release 15 for Microsoft Windows (2006).

# Results

No significant difference was found between the two groups regarding maternal age but a significant difference was found regarding gestational age at delivery, placental weight, placental thickness, neonatal birth weight and Apgar scores at 1 and 5 min (Table 1).

There was a significant difference between the two study groups regarding both abnormal uterine and umbilical artery Doppler (Table 1).

The feto-placental ratio was 5:1 in FGR group and 4.7:1 in controls. The umbilical cord was inserted eccentrically in 18 FGR cases compared to the 14 in the control group. It was noticed that by naked eye the maternal surface of the placenta of FGR cases opposing the placental bed was found markedly congested and hemorrhagic compared to the fetal surface (Fig. 2).

Microscopic features were similar in all the three zones of placenta studied. No pathological changes were observed in umbilical cord and membranes. Syncytial



Fig. 3 FGR placenta. a Chorangiosis and syncytial knots, b syncytial knots, c fibrinoid necrosis, d infarctions, e perivillous fibrinoid deposition, f calcification

knots > 30%, fibrinoid necrosis > 5%, placental infarction > 5%, perivillous fibrinoid deposition > 5% but not calcifications were significantly higher in FGR placentae (Table 1, Fig. 3).

A statistically significant (p < 0.001) increase in the expression of VEGF in FGR placentas was observed in cytotrophoblast, syncytiotrophoblast, extravillous trophoblast, vascular smooth muscle, and villous stromal and endothelial cells compared with normal term pregnancy placentas (Table 2, Fig. 4).

Regarding the assessment of MVD using CD34 for practical purposes, cases were arbitrarily into two groups: one with microvessel counts less than or equal to 90 per 200 microscopic fields (low density) and the second with microvessel counts more than 90 per 200 microscopic felids (high) (Table 3).

Accuracy of abnormal Doppler in predicting placental pathology is shown in Table 4.

Abnormal uterine artery velocimetry was significantly associated with abnormal placental pathology (Table 5).

Table 6 shows the clinical outcomes and the incidence of abnormal placental pathologies of FGR pregnancies according to uterine and umbilical artery Doppler velocimetries. Women with both abnormal uterine and umbilical artery Doppler velocimetries were delivered earlier and their babies had lower mean birth and placental weight (p < 0.001). Incidence of abnormal placental

# Fig. 4 VEGF expression in FGR placenta. a Cytotrophoblas, b syncytiotrophoblasts, c extravillous trophoblasts,

- d vascular smooth muscles,
- **e** villous stroma and endothelial cells



Table 1 The clinical characteristics of AGA and FGR pregnancies

	AGA (n = 25)	FGR $(n = 50)$	p value
Age (years)	$26.7 \pm 4.5$	$27.4 \pm 2.2$	0.338
Apgar 1 min	$8.8\pm0.47$	$7.8 \pm 1.2$	< 0.001
Apgar 5 min	$9.0\pm0.00$	$8.7\pm0.56$	0.003
Gestational age at birth (weeks)	$38.5 \pm 1.2$	$34.9 \pm 1.1$	< 0.001
Birth weight (g)	$3275\pm206$	$1815\pm237$	< 0.001
Placental weight (g)	$431 \pm 30$	$285\pm49$	< 0.001
Placenta diameter (cm)	$15.6\pm0.58$	$13.0 \pm 2.0$	< 0.001
Syncytial knots > 30%	44%	60%	< 0.001
Fibrinoid necrosis > 5%	8%	46.7%	< 0.001
Placental infarction $> 5\%$	0%	15%	< 0.001
Perivillous fibrinoid deposition $> 5\%$	1.8%	16.7%	< 0.001
calcification	56%	60%	0.121
Abnormal uterine artery Doppler	0/25 (0.0)	29/50 (58.0%)	< 0.001
Abnormal umbilical artery Doppler	0/25 (0.0)	29/50 (58.0%)	< 0.001

Data are presented as mean  $\pm$  SD

AGA appropriate for gestational age, FGR fetal growth restricted

Table 2Localization andImmunostaining intensity ofVEGF expression in placentalvillous tissues

Score	Normal pregnancy $(n = 25)$			FGR $(n = 50)$				p value	
	0	+ 1	+ 2	+ 3	0	+ 1	+ 2	+ 3	
CVSC	15 (60%)	10 (40%)	_	_	7 (14%)	6 (12%)	16 (32%)	21 (42%)	< 0.001
VCMC	15 (60%)	10 (40%)	_	_	7 (14%)	6 (12%)	16 (32%)	21 (42%)	< 0.001
VVEC	15 (60%)	10 (40%)	_	_	7 (14%)	6 (12%)	16 (32%)	21 (42%)	< 0.001
ST	15 (60%)	10 (40%)	_	_	7 (14%)	6 (12%)	16 (32%)	21 (42%)	< 0.001
СТ	15 (60%)	10 (40%)	_	_	7 (14%)	6 (12%)	16 (32%)	21 (42%)	< 0.001
EVT	15 (60%)	10 (40%)	-	_	7 (14%)	6 (12%)	16 (32%)	21 (42%)	< 0.001

Data are presented as number (%)

Staining intensity of VEGF was scored as follows: 0 (negative), weak (1+), moderate (2+), and strong (3+)

CVSC chorionic villous stromal cells, VSMC vascular smooth muscle cells, VVEC villous vascular endothelial cells, ST syncytiotrophoblasts, CT cytotrophoblasts, EVT extravillous trophoblasts

 Table 3 The result of Immunohistochemical stain of VEGF and CD34 (MVD) in FGR and AGA group

Groups	Number	CD34 (MVD)		VEGF
	Ν	Low	High	Strong + 3
AGA	25	20 (80%)	5 (20%)	0
FGR	50	5 (10%)	45 (90%)	21 (42%)
p value		< 0.001	< 0.001	< 0.001

Data are presented as number (%)

AGA appropriate for gestational age, FGR intrauterine growth restricted

pathology was significantly higher in this specific group of FGR pregnancies (p < 0.001).

## Discussion

FGR constitutes an important clinical problem associated with increased perinatal morbidity, higher incidence of neurodevelopment impairment and increased risk of adult disease, such as diabetes and cardiovascular disease [24]. Our study found major differences between placentae of FGR cases and normal controls at both gross levels (placental weight, thickness, congestion and hemorrhagic infarctions) and microscopic levels (syncytial knots, fibrinoid necrosis, placental infarctions and perivillous fibrinoid deposition).

Compared with AGA cases, FGR cases were characterized with increased number of villous infarcts villous fibrosis, increased severity of cytotrophoblast proliferation and syncytiotrophoblasts knotting, increased thickening of the villous trophoblastic basal membrane, obliterative endarteritis and perivillous fibrin deposition placenta from FGR cases with abnormal umbilical artery. Doppler velocimetries had a significantly increased number of villous infarcts, cytotrophoblast proliferation and thickening of the villous trophoblastic basal membrane than FGR cases with normal umbilical artery Doppler velocimetries depicted the accuracy of abnormal Doppler in predicting placental pathology.

We have also evaluated the effects of pre-eclampsia, per se, on the clinical and histomorphological findings of FGR pregnancies. There was no significant difference between the incidences of abnormal uterine and umbilical artery

**Table 4**Accuracy of abnormalDoppler in predicting placentalpathology

-	Sensitivity	Specificity	PPV	NPV	Overall accuracy
Uterine Doppler	100.0	71.43	82.86	100.0	88.0
Umbilical Doppler	93.55	68.42	82.86	86.67	84.0

Table 5Relationship ofabnormal uterine and umbilicalartery Doppler velocimetrieswith placental pathologies

	Abnormal uterine artery Doppler flow Placental pathology	Abnormal umbilical artery Doppler flow Placental pathology
Coefficient	21.190	1.87
Odds ratio	$2 \times 109$	6.5
95% CI	0 to $\infty$	0.73–57.8
Significance	< 0.001	0.093

**Table 6** The clinical outcomesand the incidence of abnormalplacental bed biopsy andplacental pathologies of fetalgrowth restricted pregnanciesaccording to uterine andumbilical artery Dopplervelocimetries

Umbilical artery PI	Uterine artery		Doppler flow	
	Normal		Abnormal	
	Normal	Abnormal	Normal	Abnormal
Abnormal placental pathology <sup>a</sup>	3/16 (18.8)	3/5 (60.0)	3/3 (100.0)	26/26 (100.0)
Perinatal mortality <sup>a</sup>	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Gestational age at birth (weeks)	$36.2\pm0.75$	$35.5\pm0.71$	$34.1\pm0.0$	$34.1\pm0.34$
Birth weight (g)	$2080 \pm 108$	$1970 \pm 149$	$1633 \pm 59$	$1643 \pm 122$
Placental weight (g)	$338.1\pm38.5$	$300\pm33.9$	$246.7 \pm 11.6$	$253.0\pm21.4$

Data are presented as mean  $\pm$  SD

PI Pulsatility index

<sup>a</sup>Data are presented as number (%)

Doppler velocimetries of FGR cases with and without preeclampsia (p = 0.126 and 0.067 respectively). There was also no significant difference between the incidence of abnormal placental pathology of FGR pregnancies with and without pre-eclampsia (p = 0.065 and 0.206 respectively).

The relationship between uteroplacental vascular pathology and abnormal uterine artery Doppler flow has been demonstrated by the present study as well as previous studies.

Madazli et al. [19] confirmed that abnormal Doppler velocimetry well reflect placental bed arteriopathy of pregnancies complicated by FGR.

The umbilical artery is the signature vessel in the Doppler study of the fetus as it is a direct reflection of the flow within the placenta. It is usually the first vessel to be studied when suspecting an FGR fetus.

In the present study, we found that in cases of FGR with abnormal umbilical artery Doppler velocimetry (UAD), the prevalence of placental pathology was significantly higher than those associated to normal umbilical Doppler velocimetry. The experience from many studies that in pregnancies in FGR fetuses; abnormal umbilical artery velocimetry waveforms are associated with adverse perinatal outcome [19].

The early studies that assessed uterine artery Doppler (UAD) were performed during the second trimester of gestation, between weeks 18 and 23 [25]. Most of the studies published to date agree that UAD modifications are linked to FGR but are not a reliable single predictive marker for defining a low risk category [26].

Meta-analyses have shown that umbilical artery (UA) Doppler could improve mortality and perinatal outcome in FGR [27]. This led to identify UAD as a surrogate of placental disease and consequently to consider small fetuses with normal UA Doppler as SGA with no placental disease. However, this assumption was based on false premises, because it extended observations that are valid in the most severe subset of FGR fetuses to the whole group of FGR. While UAD identifies severe placental disease, it fails to pick up instances of mild placental disease which constitute a proportion of early-onset cases and virtually all instances of late-onset FGR [28].

Considering uterine and umbilical artery velocity waveforms together, the present study as well the study of Madazli et al. [19] had demonstrated that placental bed biopsy and placental pathologies are best reflected by abnormal uterine and umbilical artery velocity waveforms, respectively. It was concluded that this finding demonstrates that uterine Doppler velocimetry reflects the maternal site and umbilical artery Doppler velocimetry reflects the placental site of fetomaternal circulation [19].

Spinillo et al. [29] identified higher incidence of abnormal placental pathologies in pregnancies with both abnormal uterine and umbilical artery Doppler velocity waveforms. They stated that all of the perinatal deaths occurred in this specific subgroup of FGR pregnancies and the most severe clinical outcomes and perinatal mortality were present when both uterine and umbilical districts were altered [29].

The accuracy of abnormal Doppler in predicting placental pathology was calculated in the present study and showed high accuracy for both uterine and umbilical artery Doppler.

Berkley et al. [30] concluded that antepartum surveillance with Doppler of the umbilical artery should be started when FGR is suspected.

Evaluation of the placenta is extremely important in attempting to understand the pathophysiology of FGR. Only with careful gross and histologic evaluation, along with clinical pathologic correlation, can the underlying causes and recurrence risks be understood.

To improve clinical practice in FGR management may therefore be found in a reappraisal of the value of placental pathology to guide maternal-fetal medicine and obstetric practice. Placenta is a documentation of events of gestational life. The placenta is one of the readily available of all human organs. The placenta, which normally has a rich vasculature, plays an important role in the development of FGR. The most common cause of FGR is placenta ischemia in which insufficient placental function results from deteriorated uteroplacental perfusion.

The placental dimensions found in the present study were significantly reduced as compared to the control group. Similar findings have been reported by Kotigwar et al. [21]. Albu et al. [17] found good correlation between reduced placental volume and low fetus weight with Umbilical Artery Doppler modifications.

Fibrinoid necrosis and perivillous fibrin deposition in intervillous space is well recognized as one of hallmarks of the placenta of FGR. Fibrinoid necrosis was observed in 47% cases in our study as compared to 32–38% cases reported by other workers [31].

Syncytial knots are indicators of compromise in fetal circulation. Incidence of syncytial knots was higher in FGR cases as compared to the control group. The Syncytial knots > 30%) were found in 60% cases which were more than the control group (44%). Similar findings have been reported by other workers [21].

The present study documents an incidence of placental infarctions (15%) similar to other workers. Authors did not find appreciable difference in prevalence of calcification in FGR and control group [11].

Madazli et al. [19] reported no pathologic abnormality of the placental bed biopsy (trophoblast invasion) in 100% of patients in the control group. While in the FGR group, the incidence of pathologic bed biopsy was 55.3% (26/47) [19].

The incomplete trophoblastic invasion (atherosis) and thrombosis of implantation site vessels may result in FGR by causing diminished blood supply to the uteroplacental unit [24].

Placental bed examination revealed no pathologic abnormality of the placental bed biopsy in the control group. However in the FGR group, the incidence of pathologic bed biopsy was 44% (22/50). This includes inadequate trophoblastic invasion (8 cases), fibrinoid necrosis of the vessel wall (acute atherosis) (6 cases), thrombosis or luminal obliteration of spiral arteries (1 case) and increased extravillous trophoblasts (7 cases). This was in agreement with the study of Madazil et al. [19].

Angiogenesis involves the branching of new microvessels from pre-existing larger blood vessels. In the present work chorangiosis (angiogenesis) was observed in 95% of FGR placenta versus 25% in the control.

The most common cause of FGR is placenta ischemia [15]. The clinical features of ischemic placental disease are revealed throughout the second half of pregnancy, but the pathophysiological processes initiating the disease originate in the first half [32].

The process of promoting the development of neovessels may be activated by chemocytokines in some pathological situations such as ischemia [33]. Structural evidence suggests that placental oxygenation is important in controlling fetoplacental angiogenesis and hence, villous differentiation [34]. Insufficient uteroplacental perfusion leading to abnormal angiogenesis may result in the pathophysiology of FGR [15].

In the present study, measurements of MVD using CD34 showed high MVD (95%) and statistically significant increase in the expression of VEGF in FGR placentas was observed in cytotrophoblast, syncytiotrophoblast, extravillous trophoblast, vascular smooth muscle, and villous stromal and endothelial cells compared with normal term pregnancy placentas. Similar findings were also reported by Barut et al. [16].

A hypoxic stimulus may lead to an excessive proliferation of villous capillaries and connective tissue via growth factors such as VEGF and FGF [35]. Abnormal vasculogenesis, angiogenesis, and pseudovasculogenesis are correlated with the impaired placental and fetal development seen in complicated pregnancies such as FGR [15]. The results from our study support the literature reporting that an alteration in placental development accompanying deteriorated angiogenesis occurs in FGR [32].

VEGF is thought to exert a dual role in the placenta, acting on both angiogenesis and trophoblast function during placental development. The increased expression of VEGF-A, that we have found in FGR placentas may promote increased endothelial cell proliferation and migration and pathological angiogenesis [32]. The observed, increased expression of CD34 and VEGF, suggests that abnormal angiogenic activity, caused by insufficient uteroplacental perfusion, results in the pathophysiology of FGR.

To the best of our knowledge, our study is the first one to evaluate both placental pathology and both umbilical and uterine artery Doppler and correlate it with neonatal outcome.

The mail limitation of our study was lack of longer follow up for developmental characteristics of FGR newborn.

#### **Compliance with Ethical Standards**

Conflict of interest No conflict of interest.

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