

Placental findings in pregnancies complicated with IUGR – histopathological and immunohistochemical analysis

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Abstract

Objective: Placental lesions and placental ischemia are typical elements of intrauterine growth restriction (IUGR). The aim of this study is to analyze histological and immunohistochemical (IHC) changes in the placentas of IUGR fetuses. **Materials and Methods:** In this prospective study, 126 placentas from small for gestational age (SGA) pregnancies (newborns with birth weight <10th percentile) that formed the study group and 31 placentas from pregnancies without SGA representing control group, were included. Placentas were examined according to standard protocol. Histopathological and IHC examinations of placentas were performed for analysis. **Results:** A certain type of lesion of placental injury is increased in placentas from SGA pregnancies. These placental lesions were placental infarction (over 5%), increased syncytial knots, intervillous fibrinoid deposition, villous thrombohematoma. Other common placental lesions were probably related to fetal adaptation to placental ischemia or represent a placental change characteristic of pregnancy evolution. **Conclusions:** It seems that although IUGR/SGA fetuses are more commonly associated with histological placental abnormalities, it cannot be established whether these abnormalities certainly contribute to IUGR, as there are no specific placental lesions in SGA placentas. Pseudo-angiomatous aspect, associated with increased syncytial knots, was specific for vascular hypoxia. Especially the magnitude of modifications of the placental structure beyond the qualitative modifications, which also lead to functional changes, are involved in this pathology of pregnancy, the onset of lesions being triggered at the level of stem villi.

Keywords: IUGR, placenta, pathology, immunohistochemistry.

Introduction

Fetal intrauterine growth and its viability depend on a vital organ – placenta – that acts for normal fetal development. Intrauterine growth restriction (IUGR) is established under the influence of fetal, maternal and placental factors that can inhibit optimal fetal growth potential. The placental factor, which due to placental insufficiency that causes an inadequate transfer of oxygen and nutrients to the fetuses, is a key element in the emergence of IUGR [1]. The impact on children born with IUGR is already known: higher postnatal cardiovascular risk, diabetes mellitus and other disorders in these newborns [2, 3], suboptimal neurodevelopment and learning difficulties at school [4, 5]. In addition to these long-term disorders, children with IUGR, or more specifically small for gestational age (SGA), represented by newborn fetal size below 10th percentile, may experience poor perinatal outcome, which sometimes leads to fetal death.

Why placental histological examination? Although there is no clear evidence of concordance between placental

lesions and clinical disorders, placental pathology can confirm clinical suspicion or provide additional information and why not be used in medical litigation in addition to adverse neonatal outcomes. The aim of this study was to evaluate placental pathological findings in pregnancies complicated by IUGR and in particular by SGA.

Materials and Methods

A number of 157 placentas were histologically and immunohistochemically evaluated from pregnancies that were followed during pregnancy in the Department of Obstetrics and Gynecology, "Filantropia" Municipal Clinical Hospital, Craiova, Romania, between October 2015–December 2017. In this prospective study, 126 placentas from SGA pregnancies (study group) and 31 placentas from pregnancies without SGA and non-complicated pregnancies (control group) were included. Placentas were examined according to standard protocol. Immediately after expulsion, the placentas were fixed with 10% formalin for 24 hours before routine embedding

in paraffin. For fixation, the placenta was cut into the multiple longitudinal slices of 1–2 cm and kept in 10% formalin for several days. After fixation, tissue samples were taken from representative areas, and the biological material was included in the paraffin.

The placenta fragments included in paraffin were sectioned with the Microm HM350 rotary microtome, equipped with a section transfer system on water bath (STS, microM). For histological examination, sections of 4- μ m thickness were stained with Hematoxylin–Eosin (HE).

To perform an immunohistochemical (IHC) examination, sections were collected on slides coated with poly-L-lysine and dried in a thermostat at 37°C for 24 hours. After this procedure, the sections were subjected to a classical protocol: deparaffinization, hydration, antigen unmasking by boiling the slides in a sodium citrate solution, pH 6, for 21 minutes (seven cycles of 3 minutes) in a microwave oven. The endogenous peroxidase blocking was performed by incubating the histological sections in 3% hydrogen peroxide for 30 minutes, at room temperature, followed by a wash in distilled water for 10 minutes and a wash in 1% phosphate-buffered saline (PBS) for five minutes. Afterwards, blocking the nonspecific sites followed by using 2% skim milk for 30 minutes. The sections were then incubated with primary antibodies, for 18 hours (over night), in a refrigerator, at 4°C. The next day, there was applied the secondary biotinylated antibody for 30 minutes, at room temperature, and then performed a washing in 1% PBS (three baths of five minutes). Then, we applied Streptavidin–Horseradish peroxidase (HRP) for 30 minutes, at room temperature, and followed by another wash in 1% PBS 3×5 minutes. The signal was detected by using 3,3'-Diaminobenzidine (DAB, Dako) and the reaction was stopped in 1% PBS. There followed the contrasting with Mayer's Hematoxylin, alcohol dehydration, xylene clarification and slide fixing using a DPX (Fluka) environment.

In our study, we used the following IHC markers: anti-CD31 (monoclonal mouse anti-human CD31, endothelial cell, clone JC70A, Dako, 1:50 dilution); anti-CD34 (monoclonal mouse anti-human CD34 Class II, clone QBEnd 10, Dako, 1:50 dilution); anti-eNOS (polyclonal antibody anti-endothelial nitric oxide synthase, clone PA3-031A, Thermo Scientific, 1:500 dilution); anti-VEGF (monoclonal mouse anti-human vascular endothelial growth factor, clone VG1, Dako, 1:100 dilution).

To classify immunoreactivity for antibodies, we used a semi-quantitative scale: 0 – negative staining; 1+ – weak staining; 2+ – moderate staining; 3+ – strong staining.

Exclusion criteria for the study group were: multiple pregnancies, fetuses with a chromosomal anomaly or congenital abnormalities, maternal infection, any maternal systemic disorder. Inclusion criteria were: singleton pregnancy, estimated fetal weight (EFW) below the 10th percentile at the routine third-trimester ultrasound examination, pregnancies with accurate gestational age dating. Neonatal outcome was evaluated by Apgar scores at 1 and 5 minutes and the need for admission to the Neonatal Intensive Care Unit (NICU). Neonatal morbidity was defined as the presence of at least one neonatal morbidity, including admission to NICU.

The management of the study was conducted in full compliance with the ethical principles contained in the “Human Rights Declaration” adopted in Helsinki, which follows the *Good Practice Rules in the Clinical Study and Legal Regulations* and with the approval of the Ethics Committee of the University of Medicine and Pharmacy of Craiova.

Results

Demographic and obstetric characteristics of patients included maternal age, parity, body mass index (BMI), smoking status, gestational age at delivery, mode of delivery. Newborn characteristics included birthweight, birthweight percentile, admission to NICU, Apgar score <7 at 1 and 5 minutes. These characteristics are presented in Table 1.

Table 1 – Maternal demographics and fetal characteristics

Characteristic	Study group	Control group
Maternal age [years]	28±6.265	28±5.686
Maternal BMI [kg/m ²]	24.3±1.926	23.6±0.899
Nulliparous	59.52%	60%
Smoking status (≥10 cigarettes/day)	15.87%	6%
Gestational age at delivery [weeks]	37.2±1.092	38.4±0.685
Premature birth (<37 weeks)	40.47%	0%
Cesarean delivery	57.93%	28%
Placental weights [g]	428.6±53.853	596.3±38.889
Birth weight [g]	2480±211.36	3280±247.686
Birth weight [percentile]	8±1.263	41±13.484
Apgar score 1 minute	7±1.11	9±0.711
Apgar score 5 minutes	8±0.914	9±0.663
Admission to NICU	34.12%	4%

Data given as mean±standard deviation (SD) or (%); BMI: Body mass index; NICU: Neonatal Intensive Care Unit.

We noticed that the average birth weight of the newborn in the study group was 2480±211.36 g, compared to 3280±247.686 g in the control group, with 8±1.263 percentiles for SGA newborn. In study group, gestational age at delivery was 37.2±1.092 weeks vs. 38.4±0.685 weeks in control group. The mean placental weights of SGA newborn were lower than those of non-SGA newborn (428.6±53.853 g vs. 596.3±38.889 g). Neonatal outcome was an Apgar score at 1 minute (7±1.11) and 5 minutes (8±0.914) in the SGA group, lower than the Apgar score at 1 and 5 minutes in the control group (9±0.711 and 9±0.663, respectively). As well, 34.12% of SGA newborn had admission to NICU.

All SGA placentas showed one or more pathological findings. The histological placental findings in SGA were varied, from not particularly interesting morphologically lesions to different degrees of uteroplacental vasculopathy. The histopathological findings which we found in the significant proportion of SGA placentas compared with normal placentas, we considered that it could contribute to the development of SGA: peri- and intervillous fibrinoid necrosis (Figure 1A), inter-/intra-/perivillous syncytial knots, vascular stasis (Figure 1B), intravillous micro-

calcifications with villous necrobiosis area (Figure 1C), placental infarction over 5% (Figure 2A), stem villi with dilated vessels each with stasis other thrombosed (Figure 2B), placental villi with dilated vessels with pseudo-angiomatous aspect (Figure 2C). Increased syncytial knots is a villous morphological abnormality specific for a hypoxic lesion that occurs by reducing utero-placental or fetoplacental flow. The same mechanism occurs in villous fibrosis, distal villous hypoplasia, reduced intervillous space, and non-specific inflammatory lesions. In the histopathological examination, a greater number of cases of infarction and calcification were noted among SGA placentas than in normal placentas. Pseudo-angiomatous aspect, associated with increased syncytial knots, was specific for vascular hypoxia. Fibrinoid necrosis, stromal fibrosis and obstructive vasculopathy were seen only in SGA placentas. Histological changes are presented in Table 2.

Table 2 – Histopathological findings

Histopathological findings	Study group	Control group
Placental infarct (over 5%)	34.12%	14%
Diffuse calcification	61.11%	32%
Increased syncytial knots	26.69%	9%
Villous thrombohematoma	19.04%	2%
Intervillous fibrinoid deposition	17.46%	12%

Angiogenesis, represented by the development of new vascular structures, is an important placental factor that is involved in IUGR promotion. Therefore, IHC markers have referred to this angiogenesis process.

The IHC study for CD31 showed a strongly positive immunoreaction in villous endothelial cells (Figure 3A), showing that CD31 may be an endothelial marker in the human placenta. The areas of intervillous fibrinoid deposition and placental infarction had a negative immunoreaction (Figure 3, B and C).

The IHC evaluation of villous tree showed a strongly positive reaction with CD34 (Figure 4A). In areas of placental infarction with villous shadows, CD34 immunoreaction was negative (Figure 4B), but the stem villi and mature intermediate villi with dilated thrombosed vessels had a strongly positive CD34 immunoreaction (Figure 4C).

Endothelial nitric oxide synthase (eNOS), the enzyme nitric oxide type III is essential for upregulation of angiogenesis, having a role in placental blood flow adaptation. eNOS immunoreaction was strongly positive at villous vascular level in the villous tree (Figure 5A) and at the level of stem villi (Figure 5B). At the level of villi that had infarct areas, eNOS immunoreaction was moderately positive at the dilated lumen of the arteriole (Figure 5C).

Vascular endothelial growth factor (VEGF) contributes and regulates the formation of new blood vessels. VEGF immunoreaction was strongly positive in the cytoplasm at the syncytiotrophoblast level in syncytial knots (Figure 6A). VEGF immunoreaction was also moderately positive at the syncytiotrophoblast level in the infarct areas (Figure 6B) and strongly positive in the syncytiotrophoblast of stem villi, mature intermediate villi and syncytial knots (Figure 6C).

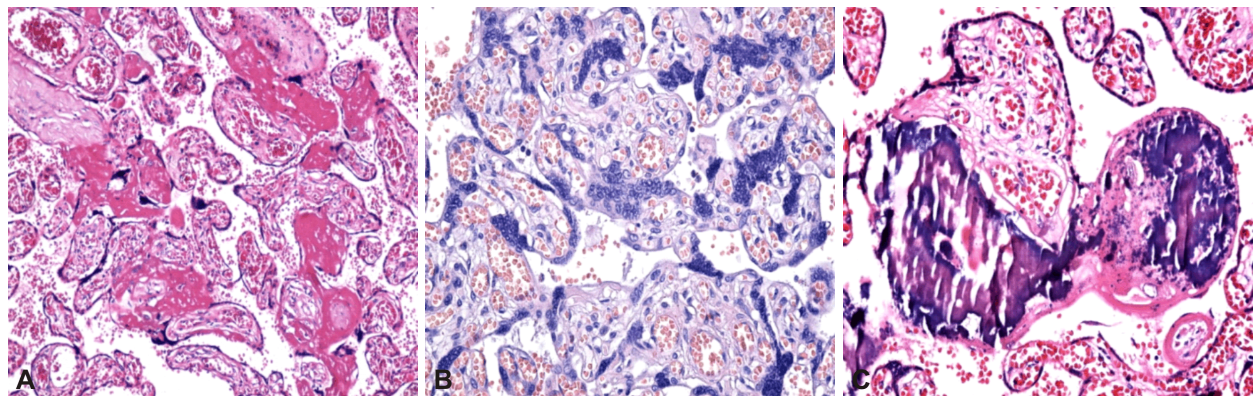


Figure 1 – (A) Peri- and intervillous fibrinoid necrosis; (B) Inter-/intra-/perivillous syncytial knots, vascular stasis; (C) Intravillous microcalcifications with villous necrobiosis area. HE staining: (A) $\times 100$; (B and C) $\times 200$.

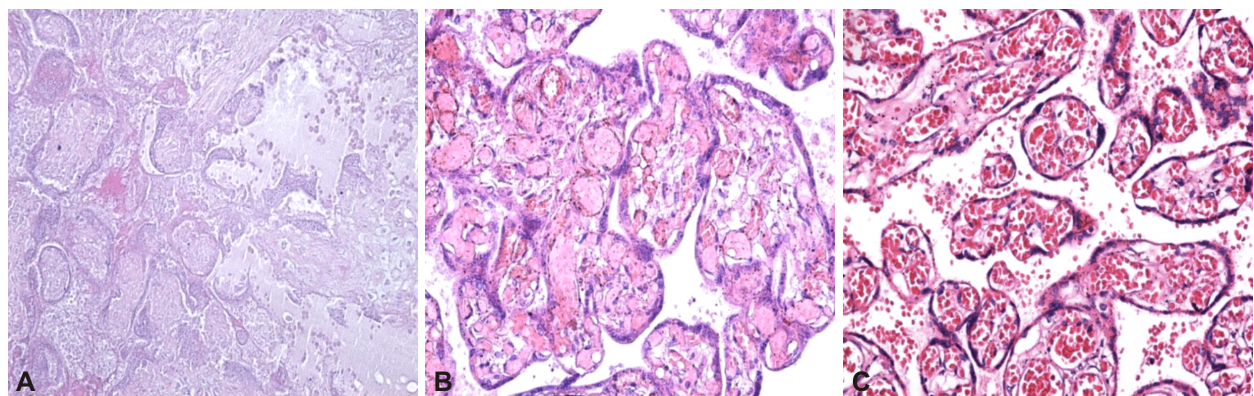


Figure 2 – (A) Placental infarction; (B) Stem villi with dilated vessels some with stasis other thrombosed; (C) Placental villi with dilated vessels with pseudo-angiomatous aspect. HE staining: (A–C) $\times 100$.

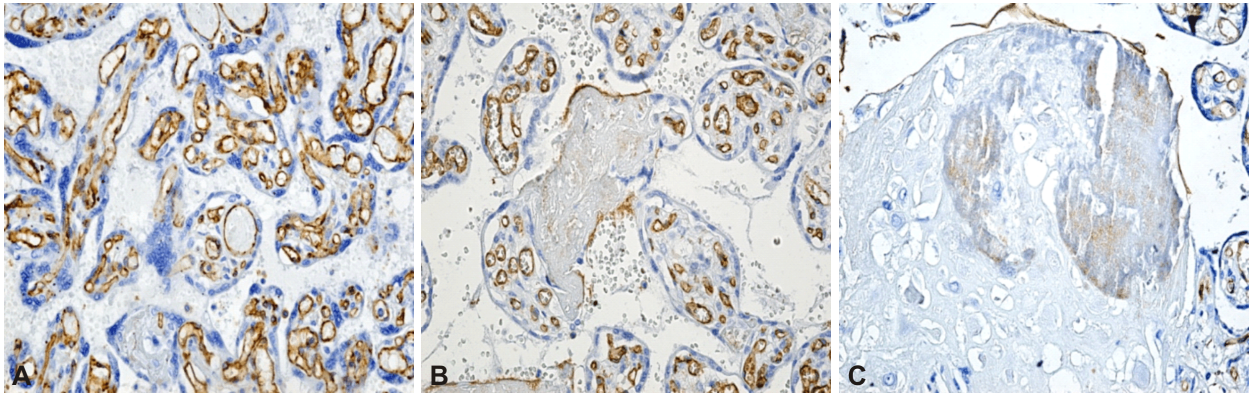


Figure 3 – (A) Stems villi, mature intermediate villi, terminal villi, syncytial knots with strongly positive CD31 immunoreaction on vascular endothelium; (B) Intervillous fibrinoid necrosis with CD31 negative immunoreaction, bounded by villi with strongly positive CD31 immunoreaction; (C) Placental infarction with microcalcifications with CD31 negative staining, together with mature intermediate villi with strongly positive CD31 immunoreaction. Anti-CD31 antibody immunomarking: (A–C) $\times 200$.

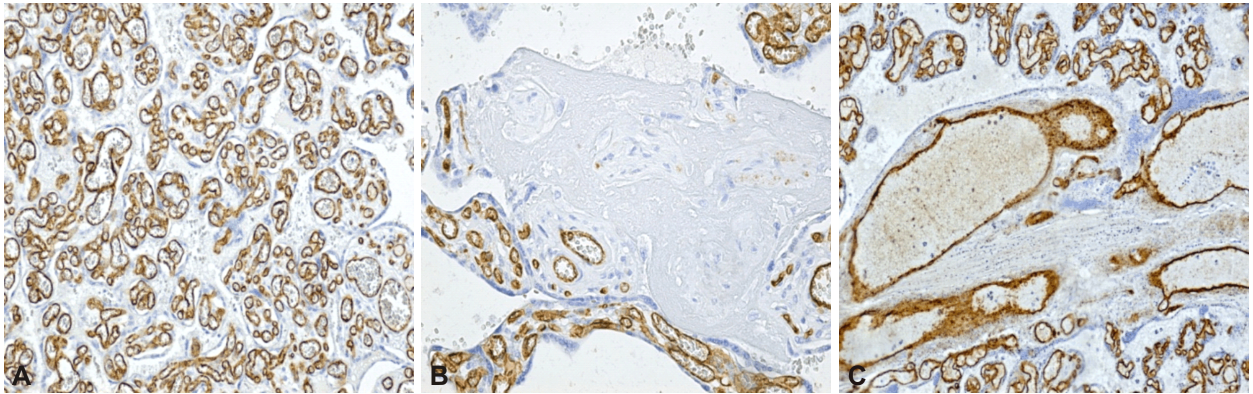


Figure 4 – (A) Villous tree with strongly positive CD31 immunoreaction; (B) Placental infarction and villous shadows with negative CD34 immunoreaction, adjacent villi with strongly positive CD34 immunoreaction; (C) Stems villi, mature intermediate villi with thrombosed dilated vessels, strongly positive CD31 immunoreaction. Anti-CD31 antibody immunomarking: (A and B) $\times 100$; (C) $\times 200$.

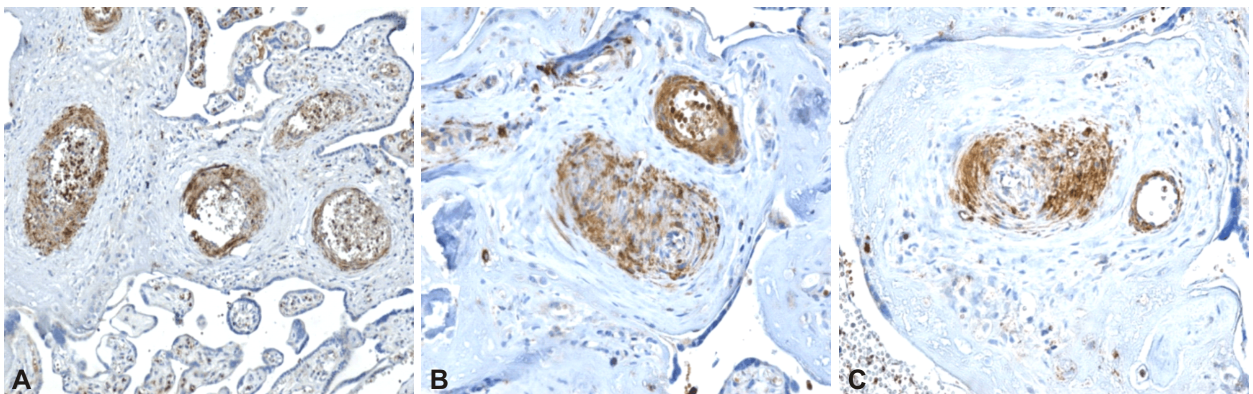


Figure 5 – (A) Villous tree with vascular strongly positive eNOS immunoreaction; (B) Stems villi with strongly positive eNOS immunoreaction at the arteriole level, surrounded by infarcted areas with microcalcifications; (C) Villous placental infarction presenting central vessels with strongly positive eNOS immunoreaction in diminished lumen arterioles and with moderately positive eNOS immunoreaction in the dilated lumen artery. Anti-eNOS antibody immunomarking: (A–C) $\times 200$. eNOS: Endothelial nitric oxide synthase.

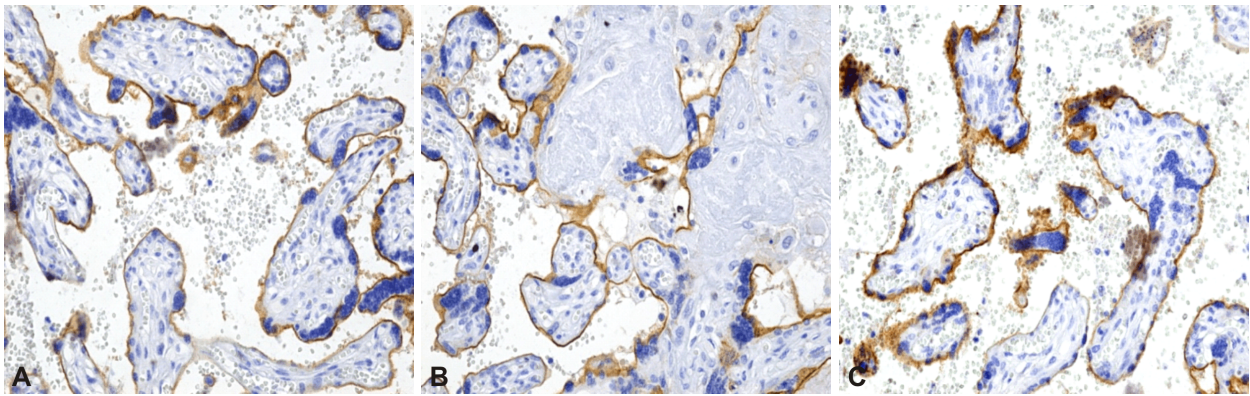


Figure 6 – (A) Syncytial knots disposed focally and isolated with strongly positive VEGF immunoreaction at cytoplasmic syncytiotrophoblast level; (B) Placental infarction area with moderately positive VEGF immunoreaction in cytoplasm of syncytiotrophoblast and in adjacent placental villi; (C) Stems villi, mature intermediate villi, syncytial knots with strongly positive VEGF immunoreaction at syncytiotrophoblast level. Anti-VEGF antibody immunomarking: (A and C) $\times 100$; (B) $\times 200$. VEGF: Vascular endothelial growth factor.

Discussions

In this study, histological and immunohistological changes in placental SGA pregnancies were investigated. The placenta may have different histological lesions that do not represent a real danger to the fetus, as happens in normal pregnancy. On the other hand, a number of cytogenetic and immunogenic factors act in maternal decidua and early placenta to develop a competent vasculogenesis and angiogenesis, thus influencing placental quality [6]. While a number of histological changes have been described in the placenta from SGA/IUGR pregnancy, no histological specific lesions to this pathology have been established [7]. However, the percentage of lesion type, placental lesions and duration of action are much more important in association with the restriction of fetal growth. Placental infarction, diffuse and irregular villous inflammations are the most common placental injuries associated with IUGR in literature [8, 9].

The relationship between placental infarction and fetal hypoxia is already demonstrated [10]. Although placental infarction can also be observed in placenta from normal pregnancies, the size and its percentage of placental injury, may affect fetal development. In our study, placental infarction (over 5%) was present at 34.12% of the study group vs. 14% of the control group. Intervillous fibrinoid deposition was more commonly observed in SGA placentas (17.46%) than in normal placentas (12%). Redline *et al.* [11] noted that the increase and expansion of intervillous fibrin deposition have shown strong correlation with placental and fetal weight. Diffuse calcification was common in both the study group (61.11%) and the control group (32%). Based on existing knowledge, it is known that diffuse calcification often occurs in mature placentas and reflects placental senescence, but there is a relationship between preterm placental calcification and adverse pregnancy outcome [12]. Iskender-Mazman *et al.* found no relationship between IUGR and diffuse dystrophic calcification [13]. The syncytial knots, which can be used to evaluate villous maturity even if they are not fully understood, have been noted in the placentas of IUGR fetuses and are assumed to be markers of placental ischemia [14]. In the present

study, we found an increase in syncytial knots, which were more common in the placentas of SGA fetuses compared to normal placentas (26.69% vs. 9%). Redline noted that women with avascular villi found in the areas of placental infarction had a higher incidence of IUGR [15, 16].

Angiogenesis is a key element in the expansion of villous vascularization and finalization of terminal villi, and IUGR occurs because of the failure of angiogenesis [17]. Placental vascular growth begins in early pregnancy and continues during gestation [18].

The expression of endothelial markers in human placentas has been extensively evaluated through immunohistochemistry [19]. In this study, we have found that CD31 showed a strongly positive immunoreactivity in villous endothelial cells, showing that CD31 may be an endothelial marker in the human placenta. The expression of CD34 immunoreactivity was positive in the stem villi and mature intermediate villi, in our study, unlike the study by Mackiewicz *et al.* [20], who found that the expression of CD34 immunoreactivity in the endothelial cells of the villous capillaries was lower in the IUGR placentas.

VEGF and eNOS are factors that take part in placental angiogenesis. eNOS immunoreaction was strongly positive at villous vascular level in the villous tree and at the level of stem villi in our study, in line with other studies [21, 22]. VEGF immunoreaction was strongly positive at the syncytiotrophoblast level, contrary to Lyall *et al.* [23] who showed a decrease in VEGF immunoreactivity in placental villi of IUGR pregnancy. However, the increase in VEGF and eNOS immunoreactivity may induce an inadequate angiogenesis [24].

This study shows that a certain types of lesions of placental injury are increased in placentas from IUGR pregnancies. These placental lesions may be placental infarction (over 5%), increased syncytial knots, intervillous fibrinoid deposition. Other common placental lesions may be related to fetal adaptation to placental ischemia.

However, a clearly defined link between the extent of these lesions and clinical outcomes has not yet been achieved.

✉ Conclusions

It seems that though SGA fetuses are more commonly associated with histological placental abnormalities, it cannot be established whether these abnormalities certainly contribute to IUGR, as there are no specific placental lesions in SGA placentas. Pseudo-angiomatous aspect associated with increased syncytial knots was specific for vascular hypoxia. Especially quantitative more than qualitative modifications of the placental structure, which also lead to functional changes, are involved in this adverse outcome of pregnancy, the onset of lesions being triggered at the level of stem villi.

Conflict of interests

The authors declare that they have no conflict of interests.

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