ORIGINAL PAPER

The influence of gestational diabetes mellitus (GDM) and gestational hypertension (GH) on placental morphological changes

ANCA-MARIA ISTRATE-OFIȚERU^{1–3)}, COSTIN BERCEANU³⁾, SABINA BERCEANU³⁾, CRISTINA JANA BUSUIOC¹⁾, GABRIELA-CAMELIA ROȘU^{1,2)}, DAMIAN DIȚESCU⁴⁾, FLORIN GROSU⁵⁾, NICOLETA-LOREDANA VOICU^{3,6)}

¹⁾Department of Histology, University of Medicine and Pharmacy of Craiova, Romania

²⁾Research Center for Microscopic Morphology and Immunology, University of Medicine and Pharmacy of Craiova, Romania

³⁾Department of Obstetrics and Gynecology, University of Medicine and Pharmacy of Craiova, Romania

⁴⁾Department of Obstetrics and Gynecology, Constantin Brâncuși University of Târgu Jiu, Romania

⁵⁾Department of Histology, Victor Papilian Faculty of Medicine, Lucian Blaga University of Sibiu, Romania

⁶⁾PhD Student, Doctoral School, University of Medicine and Pharmacy of Craiova, Romania

Abstract

Gestational diabetes mellitus (GDM) and gestational hypertension (GH) are some of the most common medical conditions associated with pregnancy. These can be correlated with placental morphopathological changes and implicitly can influence good fetal development. The age and weight of the mother can be correlated directly proportionally with those of the fetus but also with histoarchitecture and placental vascularization. The placental appearance associated with GDM and GH reveals macroscopic features, such as calcifications, fibrin deposits and placental infarcts, but the most relevant pathological features are the microscopic ones, highlighted by the classical staining techniques: Hematoxylin–Eosin (HE), Periodic Acid–Schiff (PAS)–Hematoxylin and Masson's trichrome (MT), but also by immunohistochemical technique with the help of the anti-cluster of differentiation 34 (CD34) antibody that labeled the capillary endothelium in the structure of the placental terminal villi and thus we were able to quantify the vascular density according to the associated medical pathology. The microscopic changes identified were represented by intravillous and extravillous fibrin depositions, massive placental infarctions caused by vascular suppression due to various causes, such as thrombosis, but also placental calcifications. All these macroscopic and microscopic morphopathological changes, together with the clinical data of the mother and the newborn, we have demonstrated that they are interconnected and that they can vary depending on the pathology, GH or GDM.

Keywords: placenta, chorangiosis, hypoxia, fibrin depositions.

Introduction

Worldwide, it is estimated that there are approximately 425 million adults with diabetic pathology, which represents 8.8% of the total population. In 2017, 16.2% of all mothers who gave birth to live fetuses worldwide were diagnosed during pregnancy with hyperglycemia, 86.4% with gestational diabetes mellitus (GDM) [1].

The *World Health Organization* (WHO) defines GDM as a pathology that affects pregnant women by increasing plasma glucose levels (hyperglycemia) and in this pathology several criteria are met: plasma glucose has a value above normal 7 mmol/L (126 mg/dL), and two hours after an oral loading with 75 g of glucose, it increases to 11.1 mmol/L (200 mg/dL) in the case of symptomatic GDM [2].

Maternal hyperglycemia can affect placental structure and vascularity, having a strong impact on fetal development [3, 4], with the possibility of maternal and fetal complications and increased risk of perinatal morbidity and mortality [5]. According to Pedersen [6], maternal hyperglycemia leads to increased transplacental glucose transport with the production of compensatory fetal hyperinsulinemia which leads to increased weight [6]. There are several

studies on maternal and fetal changes associated with GDM, but there has not been much research on placental changes.

High blood pressure (BP) is defined as a rise in BP >140/90 mmHg, which, if sustained, requires treatment. Regarding gestational hypertension (GH), the causes remain a mystery for the time being, but it is the most common medical disorder during pregnancy that occurs in 6–8% of pregnant women [7].

Preeclampsia (moderate GH) is thought to occur in two stages, with placental changes caused by a maternal inflammatory response. Placental aspects in normal pregnancy are characterized by the ability of the cytotrophoblast to migrate from the chorionic villi to the uterine wall, in the middle third, which it invades. At this level, the cytotrophoblast invades the spiral arteries and retrogradely replaces the endothelial layer of the maternal vessels, penetrates the myocytes and thus, the spiral arteries manage to acquire physiological properties necessary for normal placental perfusion. Unlike the arterial component, the venous component is minimally invaded. In the case of preeclampsia, the invasion of cytotrophoblasts from the interstitial space is generally superficial, and the arterial

invasion may be incomplete [8]. Through this invasion deficit there is an increase in uteroplacental vascular resistance, reducing the infusion and maternal–fetal transport of oxygen and nutrients [9–11]. Some of the placental structural changes associated with hypoxia may have effects on fetoplacental process of angiogenesis and trophoblast movements. Hypoxia can influence the process of angiogenesis through by several growth factors, ligands, and their natural antagonists [12, 13]. These factors are modified in preeclampsia and implicitly changes the placental morphology. Also, the phenomenon of hypoxia affects the processes of differentiation, proliferation, and trophoblastic recruitment, disturbing their state of equilibrium [13].

BP in the first trimester of pregnancy can drop and stay low until mid-pregnancy. A normal BP is equal to 110–120/70–80 mmHg in a healthy woman and can decrease in the first half of pregnancy by 5–10 mmHg through the phenomena of vasodilation. Systolic BP is less affected by this decrease compared to diastolic BP by the process of increasing cardiac output that compensates for vasodilatation at the systemic level. In the last quarter, BP normally reaches its initial values. High BP in pregnancy is a change in BP, reaching values of 140/90 mmHg or even higher values in a patient who was previously normotensive. These changes are associated with proteinuria (>300 mg/day) [14].

These two pathologies represent a topical topic that deserves and must be researched under different aspects, both clinical, therapeutic and morphopathological.

Aim

The aim of this research is to highlight the presence of structural and vascular changes of the placenta in the single pregnancy associated with GDM and GH. These morphopathological aspects are highlighted by histological and immunohistochemical (IHC) staining and thus it is desired to show to what extent they depend on the coexisting maternal and fetal clinical aspects.

Patients, Materials and Methods

A group of 60 patients was used for this study, women who gave birth to a single live fetus, both by eutocic and transverse segmental Caesarean section. According to the study, 30 of the 60 patients had GDM while the other 30 suffered from GH in various forms. They were all hospitalized and treated in the IInd Clinic of Obstetrics and Gynecology, Emergency County Hospital, Craiova, Romania, during 2017–2020. By using the Microsoft Excel 2010 program, we developed some statistics regarding the clinical data of the mother (age, weight) and newborns (weight, gender).

After birth, the placentae were weighed and harvested

tissue fragments. The placental tissue taken was sent to the Research Center for Microscopic Morphology and Immunology, University of Medicine and Pharmacy of Craiova, for inclusion in paraffin, sectioning, staining and analysis. With the help of formalin with a concentration of 10%, the pieces were fixed at room temperature. After this stage, the placental fragments were washed for a few hours in tap water, passed through alcohol baths with increasing concentrations, as follows: 70% (12 hours), 90%, 96%, 100% (1–2 hours each), then passed through three xylene baths (3×1 hour). After this processing step, the placental tissue fragments were introduced into the molten paraffin bath (56°C for 24 hours). The next day, solid paraffin blocks were made. The tissue thus processed was sectioned to a thickness of 5 µm using the HM350 microtome, equipped sections transfer system to water bath (STS microM), and the tissue sections were applied on simple slides and with poly-L-lysine for classical histological and IHC studies. The obtained slides were introduced one day at 37°C for drying and bonding. To visualize the placental microscopic morphology and morphopathology, both normal and modified, we used the classical stainings: Hematoxylin–Eosin (HE), Masson's trichrome (MT), and Periodic Acid Schiff (PAS)–Hematoxylin, but also the immunoreaction with the anti-cluster of differentiation 34 (CD34) for the identification and quantification of neoformation blood vessels. For both staining techniques, the xylene slides were deparaffined (3×10 minutes), water was removed from the tissue fragments by dehydration in alcohol with decreasing concentrations: 100%, 96%, 90%, 70% (5 minutes each) and the tissues were rehydrated with distilled water (dH₂O) (3×5 minutes/bath). From this moment, the protocol was different depending on the classical or IHC staining techniques. By HE staining technique, we labeled the nuclei with Hematoxylin (30 seconds) and the collagen fibers with Eosin solution (they turned pink after 2 minutes). The mucopolysaccharides and glycosaminoglycans in the placental tissue were labeled by the PAS technique, and the nuclei were also highlighted with Hematoxylin. Using the MT staining technique, the nuclei were labeled with Hematoxylin (30 seconds) and the collagen fibers in blue (20 seconds). The poly-L-lysine slides were labeled with a hydrophobic marker (DakoPen). Peri-tissular, antigenic unmasking with pH 6 citrate solution was done by boiling in the microwave (650 W, seven cycles × 3 minutes). Subsequently, the slides were washed with dH₂O (3×5 minutes), the endogenous peroxidase was inactivated with hydrogen peroxide solution (H₂O₂) of 3% concentration for 30 minutes, the slides were washed with dH₂O (2×5 minutes) for removal of H₂O₂ from tissues and with 1% phosphate-buffered saline (PBS) (1×5 minutes), non-specific sites were blocked using 3% skim milk (30 minutes). The primary antibody was dripped over the placental tissue sections (Table 1) and left to cool 4°C (for 18 hours).

Table 1 – Immunohistochemical panel of antibodies used by us

Antibody	Manufacturer	Clone	Antigenic exposure	Secondary antibody	Dilution	Labeling
Anti-CD34	Dako	QBEnd/10	Citrate, pH 6	Monoclonal mouse anti-human CD34 Class II	1:50	Neoformed blood vessels

CD34: Cluster of differentiation 34.

The next day, the slides were left for inclusion at room temperature, the primary antibody was removed by washing in 1% PBS solution (3×5 minutes), the secondary antibody was dripped (mouse VC002-025, R&D Systems, VisUCyte HRP Polymer) over placental tissue for one hour for adhesion to the primary antibody. At the end, the slides were washed in 1% PBS solution (3×5 minutes), developed with 3,3'-Diaminobenzidine (DAB) (Dako), the nuclei were labeled with Hematoxylin, the tissue water was removed by gradual dehydration in alcohols with increasing concentrations – 70%, 90%, 96%, 100% (5 minutes each), the xylene slides were clarified for 45 minutes (3×15 minutes/bath), and the slides were applied with balsam of Canada.

Results

Each pregnant woman, over 37 weeks pregnant at term, who was included in the study was not diagnosed with other conditions associated with pregnancy except those described in this research (GH and GDM). All hospitalized pregnant women were investigated clinically and imagistically, and the observation sheet presented theirs legal representative's written consent for this study based on the use of clinical aspects and macro/microscopic photographs of placentae immediately after birth and tissue pieces after processing.

GH patients were 16–46 years old, with a mean age of 28.93 ± 8.23 years, and GDM patients introduced in the study were 25–44 years old, with a mean age of 33.83 ± 6.15 years (Figure 1).

Before the birth, the 60 pregnant women were weighed, and their weight ranged between: 56–101 kg, with an average of 75.66 ± 11.5 kg for mothers with GH, and for mothers with GDM it ranged between 76–114 kg, with an average of 93.4 ± 9.3 kg (Figure 2).

Newborns included in the study were grouped as follows: 16 female and 14 male newborns with mothers presenting with GH, and 12 female and 18 male newborns with mothers presenting with GDM (Figure 3).

The weight of female newborns with mothers with GH (F-GH) ranged from 2660–3590 g, with a mean value of 2997.81 ± 293.87 g, and the weight of male newborns with mothers who presented with GH (M-GH) ranged from 2550–3900 g, with a mean value of 4295 ± 841.45 g, and the weight of female newborns with mothers who presented with GDM (F-GDM) ranged from 3760–4480 g, with a mean value of 3857 ± 161.77 g, and the weight of male newborns with mothers with GDM (M-GDM) ranged from 3695–4890 g, with a mean value of 4140.72 ± 275.31 g (Figure 4).

The placenta of newborns weighed between 443–598 g in females with mothers who presented GH (F-GH), with a mean value of 499.25 ± 49.04 g, and in male newborns with mothers who presented GH had a weight between 425–665 g, with a mean value of 549.78 ± 101.27 g. The placenta of female newborns with mothers with GDM (F-GDM) ranged from 616–745 g, with a mean value of 684.66 ± 44.21 g, and the placenta of male newborns with mothers who showed GDM (M-GDM) ranged from 615–790 g, with a mean value of 700.5 ± 47.79 g (Figure 5).

Figure 1 – The average value of age [years] of the mothers who agreed to be part of this study. The average age was significantly lower for the mothers with GH compared to the ones with GDM, $t(60) = -2.611$, $p = 0.005$. GH: Gestational hypertension; GDM: Gestational diabetes mellitus.

Figure 2 – The average value of weight [kg] of the mothers who agreed to be part of this study. The average weight was significantly lower for the mothers with GH (75.66 ± 11.55 kg) compared to the one with GDM (93.4 ± 9.33 kg), $t(60) = -6.536$, $p < 0.001$. GH: Gestational hypertension; GDM: Gestational diabetes mellitus.

Figure 3 – Gender of newborns who were part of this study. There were no significant differences in the distribution of males/females for the group of newborn mothers with GH and the group of newborn mothers with GDM, $\chi^2(n=60)=2.40379666$, $p=0.30062$. GH: Gestational hypertension; GDM: Gestational diabetes mellitus.

Figure 4 – Mean weight of fetuses dependent on the type of gestational pathology (GH/GDM) and to the newborn gender. The difference of this values was globally significant between the weight of the newborns included in these groups, $F(3, 59)=35.844$, $p<0.001$. F: Female; M: Male; GH: Gestational hypertension; GDM: Gestational diabetes mellitus.

Figure 5 – The average placental weight of the fetuses introduced in the study, depending on the type of gestational pathology (GH/GDM) and the newborn gender. The difference was globally significant between the placental weight of the newborns for these groups, $F(3, 59)=37.468$, $p<0.001$. F: Female; M: Male; GH: Gestational hypertension; GDM: Gestational diabetes mellitus.

Macroscopic morphology of the placentae from the two study groups introduced in this research (mothers with GH and mothers with GDM), but also placental morphology from the mature groups (placentae harvested from single newborns, obtained from a pregnancy unaccompanied by any pathology, a group that was approached in a previous study [15]) highlighted the aspects of the two placental components (maternal side and fetal side), the existence of amniotic membranes, the umbilical cord on the placental fetal side, but also the existence of morphopathological changes characteristic of pathology associated (Figures 6–9).

The microscopic morphological study highlighted, by means of the HE, TM and PAS–Hematoxylin usual stainings, the placental villi with the normal structure but also their modified structural aspects: the fibrin depositions located intravillous and extravillous and the intravillous infarcts. Immunohistochemistry using the anti-CD34 antibody stained brown the endothelial cells of small neoformation vessels and established that the numerical vascular density is higher in the placentae associated with GH and GDM, especially in the case of GDM,

compared to the placentae with normal structure in the control group [15] (Figures 10–12).

Through the classical HE staining technique, we identified both the structure of placental villi with normal appearance, with collagen fibers stained in pink and syncytiotrophoblast, but also the structural changes present: areas with variable fibrinoid dimensions (stained in pink). By the classical technique of PAS–Hematoxylin staining, we labeled the basement membranes of the intravillous and extravillous blood vessels and the massive fibrin depositions, rich in glycosaminoglycans and mucopolysaccharides (pink areas). Through the classical TM staining technique, we identified the placental villi, the collagen fibers (stained in blue), the placental calcifications (stained in red-purple), and the areas of intravillous and extravillous fibrin (stained in red). By immunoreaction performed with the anti-CD34 antibody, we stained the intravillous and perivillous vascular endothelium (brown label), and we noticed that their number is higher in the placental structure associated with GH or GDM, especially in GDM, compared to vascular density in the control group [15].

Figure 6 – Normal macroscopic aspects of the placenta at term, from pregnancy with a single fetus, not associated with any pathology – control group [15]: (A) Placental maternal side – amniotic membrane (white arrow), small fibrin deposits in the periphery, specific to the mature placenta (blue arrow) and normal placental villi are identified; (B) Fetal placental side – the amniotic membrane (white arrow) is identified, the umbilical cord inserted quasi-centrally and the presence of dilated blood vessels with a very large diameter. Images from the collection of Dr Anca-Maria Istrate-Ofițeru.

Figure 7 – Aspects of the placenta at term from the single pregnancy associated with GH: (A) Fetal placental side – the amniotic membrane (white arrow), the amniotic cord inserted laterally, large fibrin deposits that change the placental architecture and the presence of much dilated blood vessels (yellow arrows) are identified; (B) Placental maternal side – amniotic membrane is observed at the periphery (white arrow) and placental villi with structure modified by the presence of fibrin deposits (blue arrows). GH: Gestational hypertension. Images from the collection of Dr Anca-Maria Istrate-Ofițeru.

Figure 8 – Morphopathological aspects of the placenta associated with GH: (A) Fetal placental side – thickened, much dilated blood vessels are observed (yellow arrows) and the presence of fibrin deposits in the placental structure (blue arrows); (B) Placental maternal side – the structurally modified placental villi are observed by the presence of multiple areas with fibrin deposits (blue arrows). GH: Gestational hypertension. Images from the collection of Dr Anca-Maria Istrate-Ofiteru.

Figure 9 – Aspects of the placenta at term from the single pregnancy associated with GDM – the large size of the placenta is observed: (A) The placental maternal side – the amniotic membrane (white arrow) is identified, the placental villi with the structure modified by the presence of fibrin deposits (blue arrows); (B) Fetal placental side – the amniotic membrane at the periphery is identified (white arrow) and the placental villi with the structure modified by the presence of fibrin deposits (blue arrows). GDM: Gestational diabetes mellitus. Images from the collection of Dr Anca-Maria Istrate-Ofiteru.

Figure 10 – Microscopic characteristics of the placenta from a single pregnancy – control group [15]: (A) Placental villi with normal appearance (HE staining, $\times 200$); (B) Placental villi with normal appearance, in the structure of which the basement membranes of the blood vessels are labeled with PAS and the nuclei are labeled with Hematoxylin, and there are also small areas of fibrin deposits (stained in pink-purple) (PAS–Hematoxylin staining, $\times 200$); (C) Placental villi with normal appearance, with small perivillous and intravillous areas of fibrinoid deposits (stained in red) (MT staining, $\times 200$); (D) Immunohistochemical labeling with anti-CD34 antibody ($\times 200$) – vascular endothelium immunolabeled with brown is identified in the intravillous small blood vessels. HE: Hematoxylin–Eosin; PAS: Periodic Acid Schiff; MT: Masson's trichrome; CD34: Cluster of differentiation 34. Images from the collection of Dr Anca-Maria Istrate-Ofiteru.

Figure 11 – Microscopic features of the placenta from the single pregnancy associated with GH: (A) Placental villi with modified appearance by the presence of large, dilated blood vessels and fibrin deposits accompanied by capillary extravasation (HE staining, $\times 200$); (B) Placental villi with modified structure by the presence of intravillous, perivascular fibrin deposits labeled with PAS and the nuclei labeled with Hematoxylin (stained in pink-purple) (PAS–Hematoxylin staining, $\times 200$); (C) Placental villi with altered appearance by the presence of perivillous and intravillous fibrinoid deposits areas (stained in red) (MT staining, $\times 200$); (D) Immunohistochemical labelling with anti-CD34 antibody ($\times 200$) – immunolabeling the vascular endothelium (brown) from the intravillous small blood vessels. GH: Gestational hypertension; HE: Hematoxylin–Eosin; PAS: Periodic Acid Schiff; MT: Masson's trichrome; CD34: Cluster of differentiation 34. Images from the collection of Dr Anca-Maria Istrate-Ofiteru.

Figure 12 – Microscopic features of the placenta from the single pregnancy associated with GDM: (A) Placental villi with altered appearance due to the presence of many small blood vessels (chorangiosis) (HE staining, $\times 200$); (B) Placental villi with modified structure by the presence of massive intravillous, perivascular fibrin deposition labeled with PAS and the nuclei with Hematoxylin (stained in pink-purple) (PAS–Hematoxylin staining, $\times 200$); (C) Placental villi with altered appearance by the presence of perivillous and intravillous fibrinoid deposits areas (stained in red) and calcifications (stained in purple) (MT staining, $\times 200$); (D) Immunostaining with anti-CD34 antibody ($\times 200$) – immunolabeling of the vascular endothelium (brown) from the intravillous small blood vessels and observed and also their increased numerical density. GDM: Gestational diabetes mellitus; HE: Hematoxylin–Eosin; PAS: Periodic Acid Schiff; MT: Masson's trichrome; CD34: Cluster of differentiation 34. Images from the collection of Dr Anca-Maria Istrate-Ofiteru.

To quantify the vascular density, we took four images with the $\times 200$ magnification from the same placental section of each case (30 cases of GH-associated placenta and 30 cases of GDM-associated placenta), sections immunolabeled with anti-CD34 antibody, then we counted the small blood vessels in the structure of the placental villi, but also the perivillous vessels of each case and we made a statistical study with a comparative note using the Microsoft XL 2010 program. We obtained that the vascular density is much higher in the placentae associated with GDM by the presence of chorangiosis and slightly higher in the placentae associated with GH, compared to the control group in the previous research [15]. In the placental structure associated with GH, the vascular density was between 93.5–134.75 vessels/ $\times 200$, with a mean

value of 119.7 ± 10.48 vessels/ $\times 200$, and in the placental structure associated with GDM, the vascular density ranged from 125.5–159.25 vessels/ $\times 200$, with a mean value of 145.86 ± 7.93 vessels/ $\times 200$ (Figure 13).

We also conducted a comparative study between the mother's weight and her age, and we noticed that they could grow in a directly proportional relationship (Figure 14; Table 2). Comparing the weight of the single fetus and its placental weight, we noticed that there is also a directly proportional increase between them (Figure 15; Table 3). At the end of the clinical-statistical study, we compared the placental vascular density with the placental weight, and we highlighted the fact that there is a directly proportional relationship between them (Figure 16; Table 4).

Figure 13 – Mean vascular density from placentae of the mother with GH and GDM. The difference was globally significant between the number of blood vessels/ $\times 200$ of the newborns placentae for these groups, $F(1, 59)=118.7439$, $p<0.001$. GH: Gestational hypertension; GDM: Gestational diabetes mellitus.

Figure 14 – Comparison between average age values of the mothers in terms of gestational pathology (GH/GDM), gender of the newborns and maternal weight. It can be noticed that there is a directly proportional relationship between their values (green arrow). F: Female; M: Male; GH: Gestational hypertension; GDM: Gestational diabetes mellitus.

Table 2 – Comparison between age and weight of the mothers according to the type of gestational pathology (GH/GDM) introduced in this study

	Mother of newborn – F (GH)	Mother of newborn – M (GH)	Mother of newborn – F (GDM)	Mother of newborn – M (GDM)
<i>t Stat</i>	-8.473	-11.862	-20.631	-20.955
<i>P(T<=t) one-tail</i>	<i>p</i> <0.005	<i>p</i> <0.005	<i>p</i> <0.005	<i>p</i> <0.005

F: Female; M: Male; GH: Gestational hypertension; GDM: Gestational diabetes mellitus.

Figure 15 – Comparison between average weight value of the newborns and the average weight value of placental in terms of gestational pathology (GH/GDM). We observed that the newborns weight values and their value of placenta increase directly proportional (green star). F: Female; M: Male; GH: Gestational hypertension; GDM: Gestational diabetes mellitus.

Table 3 – Comparison between average fetal weight value and the average value of placental weight of the newborns in terms of pregnancy

	Newborn – F (GH)	Newborn – M (GH)	Newborn – F (GDM)	Newborn – M (GDM)
<i>t Stat</i>	33.544	16.085	44.009	49.820
<i>P(T<=t) one-tail</i>	<i>p</i> <0.005	<i>p</i> <0.005	<i>p</i> <0.005	<i>p</i> <0.005

F: Female; M: Male; GH: Gestational hypertension; GDM: Gestational diabetes mellitus.

Figure 16 – Comparison between average value of placental vascular density and average value of fetal weight. We observed that the fetal average value of weight increases directly proportional to the average value of placental weight (green star). In the case of placentae of mothers with GDM, an increase number of intravillous blood vessels was observed compared to the placentae of mothers with GH. F: Female; M: Male; GH: Gestational hypertension; GDM: Gestational diabetes mellitus.

Table 4 – Comparison between mean placental vascular density and fetal weight according to the type of pregnancy

	Newborn – F (GH)	Newborn – M (GH)	Newborn – F (GDM)	Newborn – M (GDM)
<i>t</i> Stat	54.116	27.936	83.109	75.916
<i>P</i> (<i>T</i> ≤ <i>t</i>) one-tail	<i>p</i> <0.005	<i>p</i> <0.005	<i>p</i> <0.005	<i>p</i> <0.005

F: Female; M: Male; GH: Gestational hypertension; GDM: Gestational diabetes mellitus.

Discussions

GDM is one of the most common medical complications associated with pregnancy, manifested by hyperglycemia with variable severity, with onset in pregnancy [16]. Usually, this pathology occurs in the second and third trimesters of pregnancy, and hyperglycemia in the first trimester is more commonly associated with pregestational diabetes.

The fetus needs for a good development of glucose, free fatty acids, and amino acids, and for them to be available it is necessary the appearance of an insulin resistance, present in normal pregnancy. Insulin resistance is a process that gradually sets in and is caused by excess weight during pregnancy and an increase in the amount of maternal and placental hormones (prolactin, cortisol, progesterone, growth factors and human lactogen hormone). Although this increases gradually, some pregnant women have normal blood sugar levels by increasing the production and release of pancreatic insulin. Pregnant women with GDM have a lower number of β -pancreatic cells, and hormone production is lower and cannot cope with pregnancy, thus appearing hyperglycemia that can have negative perinatal consequences as shown by some observational epidemiological studies [17–21].

The risk of perinatal complications increases in a relationship directly proportional to blood glucose values: fasting, postpartum glycemia or glycosylated hemoglobin values [17, 22].

Risk factors may be the mother's advanced age, weight, certain treatments that stimulated ovulation, family history, or a previous pregnancy with GDM, the latter increasing the risk of recurrence by about 50 times [23].

The effects of maternal hyperglycemia on the fetus are numerous, causing fetal hyperglycemia associated with excessive nutrition, rapid fetal growth, macrosomia, and neonatal metabolic disorders [24]. Macrosomia is associated with an increase in the number of births by Caesarean section, an increase in the number of shoulder dystocia, or other obstetric traumas. In untreated GDM patients, intrauterine fetal death may be associated with various placental morphopathological aspects. In terms of long-term consequences, children may be affected by obesity, metabolic disorders, type 2 diabetes and poor insulin tolerance and release [25].

Short-term effects on the mother may be associated with fetal weight during eutocic birth and the occurrence of obstetric trauma, or the occurrence of preeclampsia [17]. If the pregnant woman has received adequate treatment during pregnancy, the risk of childbirth is reduced by surgery or the occurrence of obstetric traumas in case of eutocic birth, but also decreases the risk of GH [26, 27].

Long-term effects may be associated with the onset of type 2 diabetes in the next 10 years [28, 29], with the occurrence of various structural changes in the heart and systemic micro/macrovacularization in the future [30, 31].

If antidiabetic treatment has not been instituted during pregnancy, associated with an adequate nutritional regimen, the placenta may undergo various structural and vascular changes. In the placenta of the third trimester, at term, mature, associated with GDM, similar to other studies, we also encountered important new changes, such as: massive depositions of intravillous, perivillous, perivascular fibrin, the presence of extensive placental infusions, calcifica-

tions, thrombosis, but most importantly, the presence of the chorangioma, or the numerical increase of the blood capillaries (>10 capillaries/terminal villi) present in the terminal villi [30–34].

Making a comparison with the study conducted by Voicu *et al.* [15], we observed that the capillary vascular density present in the terminal villi is much higher compared to the control group present in the previous study; control group represented by 30 pregnant women, without associated pathologies [15].

Daskalakis *et al.* [35] conducted a study with 40 placentae associated with GDM and obtained a percentage of 40% placentae that showed chorangiosis [35], and Huynh *et al.* [36] identified choriangioma in 38.1% of 126 placentae included in his study, associated with GH [36].

Rudge *et al.* [37] showed in their study that 12.5% of placentae associated with GDM may have placental calcifications [37].

Currently, there are a multitude of studies on angiogenesis and vascular remodeling associated with placenta in pregnancy with GDM, but no abnormalities specific to this pathology have been found, but rather pathological placental patterns. For a long time, chorangiosis has been classified as a characteristic feature of GDM, but it has now been shown to occur in other pathologies associated with pregnancy, such as GH, especially preeclampsia, or the form of moderate GH. However, the most common placental changes associated with GDM are: chorangiosis [38], placental venous immaturity [38–45]. Also, GDM is associated with accelerated microangiopathy and implicitly with capillary hypertension, causing changes in capillary permeability [46].

In the case of placentae associated with GDM, it was observed that the number of villous capillaries was increased especially in the center of the villi, which determined the increase of the distance crossed by oxygen and nutrients from the maternal to the fetal circulation [44].

The hypoxic process, along with oxygen free radicals, cytokines, inflammatory mediators, endothelial growth factor, increased hyperglycemia affect the endothelial barrier of blood vessels or angiogenic phenomena occur, with consequences on the functioning of the placenta and implicitly with consequences on fetus [12, 13, 46–49].

In this study, we observed that in the case of GDM was present the highest vascular density/ $\times 200$, labeled with the anti-CD34 antibody, compared to the vascular density of GH equal to 119.7 vessels/ $\times 200$ or with the vascular densities present in other studies, including in the case of the control group, with a single pregnancy, not associated with any pathology, where the vascular density was equal to 71.21 vessels/ $\times 200$, or with the placenta of girls in twin pregnancy where the vascular density was equal to 133.7 vessels/ $\times 200$ fetus large, 80.5 vessels/ $\times 200$ smaller fetus and 24.1 vessels/ $\times 200$ in the placental fusion area [15].

Regarding GH, we can say that preeclampsia represents the second the main reason of maternal mortality report in the world, the first place being occupied by obstetric hemorrhage. The WHO stated that 76 000 deaths may be caused by preeclampsia/year (16% of maternal mortality),

mainly associated with low socioeconomic status [50, 51]. In developed countries, this pathology is associated with premature birth, and the etiology of GH is still unknown, although more and more research is being done on this aspect, so it can be called a heterogeneous syndrome that includes several symptoms and related medical signs [52].

The incidence of preeclampsia ranges from 2–10% of all pregnancies, which is higher in underdeveloped countries, such as countries from Africa [53].

Preeclampsia dictates premature birth in order to restore normal BP, being responsible for 17% of all premature births [54].

There are a lot of risk factors associated with GH, and these include genetic, immunological, placental mass issues, pre-existing maternal conditions, infectious and nutritional factors, chronic kidney disease, but future studies may determine their true contribution [55–57].

Regarding the placental morphological aspects, we can say that through abnormal placentation a placental hypoxia can occur with consequences on the fetus. The development of the maternal–fetal placental interface is a complex process, spread throughout pregnancy [58]. The placental bed containing the spiral arteries can be altered by disrupting the process of trophoblast invasion among smooth muscle cells [59]. In preeclampsia, the extravillous trophoblast invades the uterine compartment superficially, and the invasion of the spiral arteries is also incomplete. Microscopically, the presence of fibrin depositions and syncytial nodes was frequently identified [60]. The modification of the placental architecture is more frequently associated in the case of GH with early onset, accompanied by severe manifestations (phenotype of placental preeclampsia [61].

Recent studies have shown that placentae associated with preeclampsia, intrauterine growth restriction (IUGR) have major defects in the reshaping of myometric spiral arteries that are correlated with certain clinical parameters [52].

In our study, we observed that the age and weight of the mothers with GH was higher compared to those with GDM, which may be associated risk factors. Placental weight was low in cases of GH compared to GDM, and vascular density was significantly lower compared to cases of GDM. However, comparing with the control group from the previous study [15], we can say that the placenta associated with GH has several small blood intravillous vessels, probably appeared to supplement the oxygen supply that is deficient in this pathology. Also, according to other studies, there are a number of microscopic morphopathological changes, such as placental infarction, thrombosis, massive fibrin depositions and calcifications [32–34, 61].

☐ Conclusions

Through this research we have demonstrated that there is a directly proportional link between fetal and maternal clinical aspects, as follows: the mother's age increases in direct relation to her weight but also to the fetal weight. Also, the placental mass is in a directly proportional relationship to the fetal weight, and the

number of capillary vessels, labeled with the anti-CD34 antibody, is in a directly proportional relationship to the weight of the placenta. Through the classical staining techniques, we observed the presence of different placental pathological aspects associated with GH as well as with GDM. These aspects change the placental architecture and are represented by vascular modification, the presence of calcifications, fibrin depositions, the presence of vascular thrombosis, placental infarction, and all these influence fetal growth and development but also the normal evolution of pregnancy.

Conflict of interests

The authors declare that they have no conflict of interests.

Acknowledgments

Microscopic images have been acquired in the Research Center for Microscopic Morphology and Immunology, University of Medicine and Pharmacy of Craiova, Romania (Manager: Professor Laurențiu Mogoantă, MD, PhD).

Authors' contribution

Anca-Maria Istrate-Ofițeru and Costin Berceanu equally contributed to this article.

References

- [1] Cho NH, Shaw JE, Karuranga S, Huang Y, da Rocha Fernandes JD, Ohlrogge AW, Malanda B. IDF Diabetes Atlas: global estimates of diabetes prevalence for 2017 and projections for 2045. *Diabetes Res Clin Pract*, 2018, 138: 271–281. <https://doi.org/10.1016/j.diabres.2018.02.023> PMID: 29496507
- [2] ***. Diagnostic criteria and classification of hyperglycaemia first detected in pregnancy: a World Health Organization Guideline. *Diabetes Res Clin Pract*, 2014, 103(3):341–363. <https://doi.org/10.1016/j.diabres.2013.10.012> PMID: 24847517
- [3] Huynh J, Dawson D, Roberts D, Bentley-Lewis R. A systematic review of placental pathology in maternal diabetes mellitus. *Placenta*, 2015, 36(2):101–114. <https://doi.org/10.1016/j.placenta.2014.11.021> PMID: 25524060 PMCID: PMC4339292
- [4] Ladfors L, Shaat N, Wiberg N, Katasarou A, Berntorp K, Kristensen K. Fetal overgrowth in women with type 1 and type 2 diabetes mellitus. *PLoS One*, 2017, 12(11):e0187917. <https://doi.org/10.1371/journal.pone.0187917> PMID: 29121112 PMCID: PMC5679529
- [5] Nelson SM, Coan PM, Burton GJ, Lindsay RS. Placental structure in type 1 diabetes: relation to fetal insulin, leptin, and IGF-I. *Diabetes*, 2009, 58(11):2634–2641. <https://doi.org/10.2337/db09-0739> PMID: 19690062 PMCID: PMC2768170
- [6] Pedersen J. Diabetes and pregnancy: blood sugar of newborn infants during fasting and glucose administration. *Ugeskr Laeger*, 1952, 114(21):685. PMID: 14958933
- [7] ***. Report of the National High Blood Pressure Education Program Working Group on high blood pressure in pregnancy. *Am J Obstet Gynecol*, 2000, 183(1):S1–S22. PMID: 10920346
- [8] Fisher SJ. Why is placentation abnormal in preeclampsia? *Am J Obstet Gynecol*, 2015, 213(4 Suppl):S115–S122. <https://doi.org/10.1016/j.ajog.2015.08.042> PMID: 26428489 PMCID: PMC4592742
- [9] Kattah AG, Garovic VD. The management of hypertension in pregnancy. *Adv Chronic Kidney Dis*, 2013, 20(3):229–239. <https://doi.org/10.1053/j.ackd.2013.01.014> PMID: 23928387 PMCID: PMC3925675
- [10] Lim KH, Zhou Y, Janatpour M, McMaster M, Bass K, Chun SH, Fisher SJ. Human cytotrophoblast differentiation/invasion is abnormal in pre-eclampsia. *Am J Pathol*, 1997, 151(6):1809–1818. PMID: 9403732 PMCID: PMC1858365
- [11] Huppertz B, Peeters LLH. Vascular biology in implantation and placentation. *Angiogenesis*, 2005, 8(2):157–167. <https://doi.org/10.1007/s10456-005-9007-8> PMID: 16211358
- [12] Charnock-Jones DS, Kaufmann P, Mayhew TM. Aspects of human fetoplacental vasculogenesis and angiogenesis. I. Molecular regulation. *Placenta*, 2004, 25(2–3):103–113. <https://doi.org/10.1016/j.placenta.2003.10.004> PMID: 14972443
- [13] Mayhew TM, Charnock-Jones DS, Kaufmann P. Aspects of human fetoplacental vasculogenesis and angiogenesis. III. Changes in complicated pregnancies. *Placenta*, 2004, 25(2–3): 127–139. <https://doi.org/10.1016/j.placenta.2003.10.010> PMID: 14972445
- [14] Moser M, Brown CM, Rose CH, Garovic VD. Hypertension in pregnancy: is it time for a new approach to treatment? *J Hypertens*, 2012, 30(6):1092–1100. <https://doi.org/10.1097/HJH.0b013e3283536319> PMID: 22573074 PMCID: PMC3746762
- [15] Voicu NL, Berceanu S, Paitici Ș, Roșu GC, Iovan L, Berceanu C, Bohlîțea RE, Istrate-Ofițeru AM. Clinical and morphological study of single and twin pregnancies placenta. *Curr Health Sci J*, 2020, 46(1):44–55. <https://doi.org/10.12865/CHSJ.46.01.07> PMID: 32637165 PMCID: PMC7323729
- [16] Metzger BE, Coustan DR. Summary and recommendations of the Fourth International Workshop-Conference on Gestational Diabetes Mellitus. The Organizing Committee. *Diabetes Care*, 1998, 21(Suppl 2):B161–B167. PMID: 9704245
- [17] HAPO Study Cooperative Research Group; Metzger BE, Lowe LP, Dyer AR, Trimble ER, Chaovarindr U, Coustan DR, Hadden DR, McCance DR, Hod M, McIntyre HD, Oats JJ, Persson B, Rogers MS, Sacks DA. Hyperglycemia and adverse pregnancy outcomes. *N Engl J Med*, 2008, 358(19):1991–2002. <https://doi.org/10.1056/NEJMoa0707943> PMID: 18463375
- [18] Sacks DA, Greenspoon JS, Abu-Fadil S, Henry HM, Wolde-Tsadiq G, Yao JF. Toward universal criteria for gestational diabetes: the 75-gram glucose tolerance test in pregnancy. *Am J Obstet Gynecol*, 1995, 172(2 Pt 1):607–614. [https://doi.org/10.1016/0002-9378\(95\)90580-4](https://doi.org/10.1016/0002-9378(95)90580-4) PMID: 7856693
- [19] Sermer M, Naylor CD, Gare DJ, Kenshole AB, Ritchie JW, Farine D, Cohen HR, McArthur K, Holzapfel S, Biringier A, Chen E; The Toronto Tri-Hospital Gestational Diabetes Investigators. Impact of increasing carbohydrate intolerance on maternal-fetal outcomes in 3637 women without gestational diabetes. The Toronto Tri-Hospital Gestational Diabetes Project. *Am J Obstet Gynecol*, 1995, 173(1):146–156. [https://doi.org/10.1016/0002-9378\(95\)90183-3](https://doi.org/10.1016/0002-9378(95)90183-3) PMID: 7631672
- [20] Jensen DM, Damm P, Sørensen B, Mølsted-Pedersen L, Westergaard JG, Klebe J, Beck-Nielsen H. Clinical impact of mild carbohydrate intolerance in pregnancy: a study of 2904 nondiabetic Danish women with risk factors for gestational diabetes mellitus. *Am J Obstet Gynecol*, 2001, 185(2):413–419. <https://doi.org/10.1067/mob.2001.115864> PMID: 11518901
- [21] Schmidt MI, Duncan BB, Reichelt AJ, Branchtein L, Matos MC, Costa e Forti A, Spichler ER, Pousada JM, Teixeira MM, Yamashita T; Brazilian Gestational Diabetes Study Group. Gestational diabetes mellitus diagnosed with a 2-h 75-g oral glucose tolerance test and adverse pregnancy outcomes. *Diabetes Care*, 2001, 24(7):1151–1155. <https://doi.org/10.2337/diacare.24.7.1151> PMID: 11423494
- [22] Lowe LP, Metzger BE, Dyer AR, Lowe J, McCance DR, Lappin TR, Trimble ER, Coustan DR, Hadden DR, Hod M, Oats JJ, Persson B; HAPO Study Cooperative Research Group. Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study: associations of maternal A1C and glucose with pregnancy outcomes. *Diabetes Care*, 2012, 35(3):574–580. <https://doi.org/10.2337/dc11-1687> PMID: 22301123 PMCID: PMC3322718
- [23] Syngelaki A, Pastides A, Kotecha R, Wright A, Akolekar R, Nicolaides KH. First-trimester screening for gestational diabetes mellitus based on maternal characteristics and history. *Fetal Diagn Ther*, 2015, 38(1):14–21. <https://doi.org/10.1159/000369970> PMID: 25531073
- [24] Diabetes Canada Clinical Practice Guidelines Expert Committee; Feig DS, Berger H, Donovan L, Godbout A, Kader T, Keely E, Sanghera R. Diabetes and pregnancy. *Can J Diabetes*, 2018, 42(Suppl 1):S255–S282. <https://doi.org/10.1016/j.cjcd.2017.10.038> PMID: 29650105
- [25] Falavigna M, Schmidt MI, Trujillo J, Alves LF, Wendland ER, Torloni MR, Colagiuri S, Duncan BB. Effectiveness of gestational diabetes treatment: a systematic review with quality of evidence assessment. *Diabetes Res Clin Pract*, 2012,

- 98(3):396–405. <https://doi.org/10.1016/j.diabres.2012.09.002> PMID: 23031412
- [26] Damm P, Houshmand-Oeregaard A, Kelstrup L, Lauenborg J, Mathiesen ER, Clausen TD. Gestational diabetes mellitus and long-term consequences for mother and offspring: a view from Denmark. *Diabetologia*, 2016, 59(7):1396–1399. <https://doi.org/10.1007/s00125-016-3985-5> PMID: 27174368
 - [27] Crowther CA, Hiller JE, Moss JR, McPhee AJ, Jeffries WS, Robinson JS; Australian Carbohydrate Intolerance Study in Pregnant Women (ACHOIS) Trial Group. Effect of treatment of gestational diabetes mellitus on pregnancy outcomes. *N Engl J Med*, 2005, 352(24):2477–2486. <https://doi.org/10.1056/NEJMoa042973> PMID: 15951574
 - [28] Kim C, Newton KM, Knopp RH. Gestational diabetes and the incidence of type 2 diabetes: a systematic review. *Diabetes Care*, 2002, 25(10):1862–1868. <https://doi.org/10.2337/diacare.25.10.1862> PMID: 12351492
 - [29] Bellamy L, Casas JP, Hingorani AD, Williams D. Type 2 diabetes mellitus after gestational diabetes: a systematic review and meta-analysis. *Lancet*, 2009, 373(9677):1773–1779. [https://doi.org/10.1016/s0140-6736\(09\)60731-5](https://doi.org/10.1016/s0140-6736(09)60731-5) PMID: 19465232
 - [30] Istrătoae O, Ofițeru AM, Nicola GC, Radu RI, Florescu C, Mogoantă L, Streba CT. Myocardial interstitial fibrosis – histological and immunohistochemical aspects. *Rom J Morphol Embryol*, 2015, 56(4):1473–1480. PMID: 26743297
 - [31] Istrătoae O, Pirici I, Ofițeru AM, Grosu F, Brînzea A, Olar L, Efreim IC. Evaluation of cardiac microvasculature in patients with diffuse myocardial fibrosis. *Rom J Morphol Embryol*, 2016, 57(4):1351–1356. PMID: 28174803
 - [32] Berceanu C, Tetileanu AV, Ofițeru AM, Brătilă E, Mehedințu C, Voicu NL, Szasz FA, Berceanu S, Vlădăreanu S, Navolan DB. Morphological and ultrasound findings in the placenta of diabetic pregnancy. *Rom J Morphol Embryol*, 2018, 59(1): 175–186. PMID: 29940626
 - [33] Berceanu C, Ciurea EL, Cirstoiu MM, Berceanu S, Ofițeru AM, Mehedințu C, Berbece SI, Ciortea R, Stepan AE, Balseanu TA. Maternal-fetal management in thrombophilia related and placenta-mediated pregnancy complications. *Rev Chim (Bucharest)*, 2018, 69(9):2396–2401. <https://doi.org/10.37358/RC.18.9.6541>
 - [34] Pătru CL, Marinaș MC, Tudorache Ș, Căpitănescu RG, Sîrbu OG, Zorilă GL, Cernea N, Istrătoae O, Ofițeru AM, Roșu GC, Iovan L, Iliescu DG. The performance of hyperadherence markers in anterior placenta praevia overlying the Caesarean scar. *Rom J Morphol Embryol*, 2019, 60(3):861–867. PMID: 31912097
 - [35] Daskalakis G, Marinopoulos S, Krieles V, Papapanagiotou A, Papantoniou N, Mesogitis S, Antsaklis A. Placental pathology in women with gestational diabetes. *Acta Obstet Gynecol Scand*, 2008, 87(4):403–407. <https://doi.org/10.1080/00016340801908783> PMID: 18382864
 - [36] Huynh J, Yamada J, Beauharnais C, Wenger JB, Thadhani RI, Wexler D, Roberts DJ, Bentley-Lewis R. Type 1, type 2 and gestational diabetes mellitus differentially impact placental pathologic characteristics of uteroplacental malperfusion. *Placenta*, 2015, 36(10):1161–1166. <https://doi.org/10.1016/j.placenta.2015.08.004> PMID: 26303757 PMCID: PMC4609602
 - [37] Rudge MVC, Lima CP, Damasceno DC, Sinzato YK, Napoli G, Rudge CVC, Gallego FQ, Calderon IMP. Histopathological placental lesions in mild gestational hyperglycemic and diabetic women. *Diabetol Metab Syndr*, 2011, 3(1):19. <https://doi.org/10.1186/1758-5996-3-19> PMID: 21831283 PMCID: PMC3174871
 - [38] Roberts DJ, Raspollini MR. Histopathology of placenta. In: Hod M, Jovanovic LG, Di Renzo GC, De Leiva A, Langer O (eds). *Textbook of diabetes and pregnancy*. 2nd edition, Informa Healthcare, UK, 2008, 41–46. <https://doi.org/10.1201/9781003039976>
 - [39] Stanek J. Chorangiomas of chorionic villi: what does it really mean? *Arch Pathol Lab Med*, 2016, 140(6):588–593. <https://doi.org/10.5858/arpa.2015-0160-OA> PMID: 27232351
 - [40] Ogino S, Redline RW. Villous capillary lesions of the placenta: distinctions between chorangioma, chorangiomatosis, and chorangiomas. *Hum Pathol*, 2000, 31(8):945–954. <https://doi.org/10.1053/hupa.2000.9036> PMID: 10987255
 - [41] Soma H, Murai N, Tanaka K, Oguro T, Kokuba H, Fujita K, Mineo S. Angiogenesis in villous chorangiomas observed by ultrastructural studies. *Med Mol Morphol*, 2013, 46(2):77–85. <https://doi.org/10.1007/s00795-013-0010-7> PMID: 23446359
 - [42] Schwartz DA. Chorangiomas and its precursors: underdiagnosed placental indicators of chronic fetal hypoxia. *Obstet Gynecol Surv*, 2001, 56(9):523–525. <https://doi.org/10.1097/00006254-200109000-00001> PMID: 11524618
 - [43] Gupta R, Nigam S, Arora P, Khurana N, Batra S, Mandal AK. Clinico-pathological profile of 12 cases of chorangiomas. *Arch Gynecol Obstet*, 2006, 274(1):50–53. <https://doi.org/10.1007/s00404-005-0076-0> PMID: 16208478
 - [44] Rossi R, Scillitani G, Vimercati A, Fiore MG, Mastrodonato M, Resta L. Diabetic placenta: ultrastructure and morphometry of the term villi. *Anal Quant Cytopathol Histopathol*, 2012, 34(5):239–247. PMID: 23301383
 - [45] De La Ossa MM, Cabello-Inchausti B, Robinson MJ. Placental chorangiomas. *Arch Pathol Lab Med*, 2001, 125(9):1258. [https://doi.org/10.1043/0003-9985\(2001\)125<1258:PC>2.0.CO;2](https://doi.org/10.1043/0003-9985(2001)125<1258:PC>2.0.CO;2) PMID: 11520290
 - [46] Leach L, Mayhew TM. Vasculogenesis and angiogenesis in the diabetic placenta. In: Djelmiš J, Desoye G, Ivanišević M (eds). *Diabetology of pregnancy*. Book Series: "Frontiers in Diabetes", vol. 17 – Porta M, Matschinsky FM (eds), Karger, Basel, 2005, 110–126. <https://doi.org/10.1159/isbn.978-3-318-01214-9>
 - [47] Zygmunt M. Placental circulation: clinical significance. *Early Pregnancy*, 2001, 5(1):72–73. PMID: 11753521
 - [48] Kaufmann P, Mayhew TM, Charnock-Jones DS. Aspects of human fetoplacental vasculogenesis and angiogenesis. II. Changes during normal pregnancy. *Placenta*, 2004, 25(2–3): 114–126. <https://doi.org/10.1016/j.placenta.2003.10.009> PMID: 14972444
 - [49] Helske S, Vuorela P, Carpen O, Hornig C, Weich H, Halmesmaki E. Expression of vascular endothelial growth factor receptors 1, 2 and 3 in placentas from normal and complicated pregnancies. *Mol Hum Reprod*, 2001, 7(2):205–210. <https://doi.org/10.1093/molehr/7.2.205> PMID: 11160848
 - [50] Ronsmans C, Graham WJ; Lancet Maternal Survival Series steering group. Maternal mortality: who, when, where, and why. *Lancet*, 2006, 368(9542):1189–1200. [https://doi.org/10.1016/S0140-6736\(06\)69380-X](https://doi.org/10.1016/S0140-6736(06)69380-X) PMID: 17011946
 - [51] Khan KS, Wojdyla D, Say L, Gülmezoglu AM, Van Look PF. WHO analysis of causes of maternal death: a systematic review. *Lancet*, 2006, 367(9516):1066–1074. [https://doi.org/10.1016/S0140-6736\(06\)68397-9](https://doi.org/10.1016/S0140-6736(06)68397-9) PMID: 16581405
 - [52] Duley L. The global impact of pre-eclampsia and eclampsia. *Semin Perinatol*, 2009, 33(3):130–137. <https://doi.org/10.1053/j.semper.2009.02.010> PMID: 19464502
 - [53] Ananth CV, Vintzileos AM. Epidemiology of preterm birth and its clinical subtypes. *J Matern Fetal Neonatal Med*, 2006, 19(12):773–782. <https://doi.org/10.1080/14767050600965882> PMID: 17190687
 - [54] Piccoli GB, Zakharova E, Attini R, Ibarra Hernandez M, Orozco Guillien A, Alrukhaimi M, Liu ZH, Ashuntantang G, Covella B, Cabiddu G, Li PKT, Garcia-Garcia G, Levin A. Pregnancy in chronic kidney disease: need for higher awareness. A pragmatic review focused on what could be improved in the different CKD stages and phases. *J Clin Med*, 2018, 7(11):415. <https://doi.org/10.3390/jcm7110415> PMID: 30400594 PMCID: PMC6262338
 - [55] Hirose N, Ohkuchi A, Usui R, Matsubara S, Suzuki M. Risk of preeclampsia in women with CKD, dialysis or kidney transplantation. *Med J Obstet Gynecol*, 2014, 2(2):1028. <https://www.jsimedcentral.com/Obstetrics/obstetrics-sp1d-prediction-preeclampsia-1028.pdf>
 - [56] Williams PJ, Morgan L. The role of genetics in pre-eclampsia and potential pharmacogenomic interventions. *Pharmacogenomics Pers Med*, 2012, 5:37–51. <https://doi.org/10.2147/PGPM.S23141> PMID: 23226061 PMCID: PMC3513227
 - [57] Maltepe E, Bakardjiev AI, Fisher SJ. The placenta: transcriptional, epigenetic, and physiological integration during development. *J Clin Invest*, 2010, 120(4):1016–1025. <https://doi.org/10.1172/JCI41211> PMID: 20364099 PMCID: PMC2846055
 - [58] Brosens IA, Robertson WB, Dixon HG. The role of the spiral arteries in the pathogenesis of preeclampsia. *Obstet Gynecol Annu*, 1972, 1:177–191. PMID: 4669123

- [59] Redman CWG, Tannetta DS, Dragovic RA, Gardiner C, Southcombe JH, Collett GP, Sargent IL. Review: does size matter? Placental debris and the pathophysiology of pre-eclampsia. *Placenta*, 2012, 33(Suppl):S48–S54. <https://doi.org/10.1016/j.placenta.2011.12.006> PMID: 22217911
- [60] Lyall F, Robson SC, Bulmer JN. Spiral artery remodeling and trophoblast invasion in preeclampsia and fetal growth restriction: relationship to clinical outcome. *Hypertension*, 2013, 62(6):1046–1054. <https://doi.org/10.1161/HYPERTENSIONAHA.113.01892> PMID: 24060885
- [61] Berceanu C, Mehedințu C, Berceanu S, Voicu NL, Brătilă E, Istrate-Ofițeru AM, Navolan DB, Niculescu M, Szasz FA, Căpitănescu RG, Văduva CC. Morphological and ultrasound findings in multiple pregnancy placentation. *Rom J Morphol Embryol*, 2018, 59(2):435–453. PMID: 30173248

Corresponding authors

Gabriela-Camelia Roșu, Junior Assistant, MD, Department of Histology, University of Medicine and Pharmacy of Craiova, 2 Petru Rareș Street, 200349 Craiova, Dolj County, Romania; Phone +40766–313 097, e-mail: nicola_camelia92@yahoo.com

Florin Grosu, MD, PhD, Department of Histology, Victor Papilian Faculty of Medicine, Lucian Blaga University of Sibiu, 10 Victoriei Avenue, 550024 Sibiu, Romania; Phone +40746–097 966, e-mail: drfloringrosu@gmail.com

Received: January 30, 2020

Accepted: September 23, 2020