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Evaluation of placental vascularization in thrombophilia and intrauterine growth restriction (IUGR)

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Abstract

The placenta is an essential organ in the proper development of pregnancy, and it can present a lot of structural and vascular lesions that can affect fetal development. One of the pathologies associated with pregnancy, which can change the placental structure is thrombophilia (TPh), and this can be correlated with an intrauterine growth restriction (IUGR) of the fetus. Maternal clinical aspects (age, weight) can be correlated with fetal ones (weight, gender), but also with the structural and vascular aspect of the placenta. The placental structure associated with TPh and IUGR shows macroscopic changes, such as fibrin deposition, calcifications and placental infarctions, but microscopic lesions are best highlighted by classical staining techniques: Hematoxylin–Eosin (HE), Masson's trichrome (MT) and Periodic Acid–Schiff (PAS)–Hematoxylin, but also by immunohistochemistry technique with the help of anti-cluster of differentiation 34 (CD34) antibody that could make it possible to quantify vascular density depending on the pathology. Microscopic changes were massive infarcts caused by vascular ischemia, intravenous and extravillous fibrin deposits, calcifications, and vascular thrombosis. All these clinical, morphological and morphopathological data are interconnected and may vary in the presence of TPh and IUGR.

Keywords: placenta, vascularization, infarcts, fibrin depositions.

Introduction

Thrombophilia (TPh) and intrauterine growth restriction (IUGR) can significantly influence fetal growth and development. Intrauterine fetal growth is caused by placental aspects. The placenta is an intensely vascularized organ, involved in fetal growth, but also in the transport of various nutrients, hormones, or substances [1, 2]. During placental development, the blastocyst attaches to the maternal lumen and evolves into a syncytiotrophoblast and cytotrophoblast. The primary placental villi have a branched appearance, and from their level the secondary and tertiary villi are formed, which achieve the utero-placental circulation. As pregnancy develops, the process of angiogenesis intensifies [3, 4].

IUGR can occur under the influence of certain maternal, placental, or fetal factors. When the placenta undergoes various structural and vascular changes, it becomes deficient in the mechanism of transfer of oxygen and substances necessary for fetal development, so IUGR can be installed, with various disorders that can be associated, in the long term: cardiovascular disease, diabetes or learning disorders, and according to gestational age, newborns are younger than the appropriate age and may be below the 10th percentile, with sometimes major repercussions on their development [5–10].

During placental development, various changes can occur, such as angiogenesis abnormalities, remodeling of the spiral arteries, the appearance of placental infarctions, fibrin deposits or calcifications, but also the abnormal invasion of the trophoblast [5]. Frequently, these placental changes have been associated with the presence of hereditary maternal TPh [11], which led to the finding of a link between this and insufficient fetal development [12]. TPh has been shown over the years to be associated with structural and vascular placental changes [13]. The most common placental changes present in TPh and identified in classical staining are thrombosis, villous infarctions, calcifications and massive fibrinoid necrosis [14, 15], and immunohistochemical staining has shown that placental vascularity is numerically low in both pathologies. These changes in placental architecture may be the cause of IUGR and other changes in fetal development.

Aim

The aim of this research is to identify the presence of structural and vascular placental changes in both TPh and IUGR, through classical and immunohistochemical

staining, but also to correlate them with maternal and fetal clinical aspects.

Patients, Materials and Methods

This research was performed on a group of 60 patients who gave birth to a live fetus, both eutocic and by segmental transverse Caesarean section (STCS). Of these, 30 patients without TPh changes gave birth to a live fetus with IUGR, and another 30 patients were diagnosed with TPh. All of these patients were hospitalized in the IInd Clinic of Obstetrics and Gynecology, Emergency County Hospital, Craiova, Romania, during 2017–2020. With the help of the Microsoft Excel 2010 program, a clinical-statistical study was performed which was based on the clinical characteristics of the mother (age, weight), but also of the newborns (weight, gender).

After birth, the macroscopic aspects of the placentae were analyzed, the placentae were weighed, and tissue fragments were collected. These placental fragments were sent to the Research Center for Microscopic Morphology and Immunology (University of Medicine and Pharmacy of Craiova) for processing and analysis. Tissue fragments were held for fixation in 10% neutral buffered formalin, at room temperature. After fixation, the placental tissue was washed abundantly in tap water for several hours, passed through successive ethanol baths with increasing concentrations [70% (12 hours), 90%, 96%, and 100% (1–2 hours each)], then through three baths of xylene (3×1 hour). Finally, the tissue fragments were introduced into molten paraffin (56°C overnight, until the next day) and embedded in solid paraffin blocks. The tissue included in the paraffin blocks was sectioned using the HM350 microtome, at a thickness of 5 μm, equipped with a sections transfer system to water bath (STS, MicroM), and placental sections were applied on single slides for classical histological studies and on poly-L-lysine slides for immunohistochemical studies. The obtained slides were stored for 24 hours, at 37°C, overnight, for drying and bonding. To visualize the normal placental structure, as well as the modified placental structure, we used the classical stainings: Hematoxylin–Eosin (HE), Masson's trichrome (MT) and Periodic Acid Schiff (PAS)–Hematoxylin, but also immunohistochemical technique to identify and quantify neoformation vessels. For all the stainings, we deparaffined the slides in three xylene baths (3×10 minutes), we removed the water from the tissues by dehydration in ethanol baths with decreasing concentrations [100%, 96%, 90%, and 70% (5 minutes each)] and we rehydrated the tissues with distilled water (dH_2O) for 15 minutes. From this point, the protocol varied depending on the staining, classical or immunohistochemical. For HE staining, we marked the nuclei with Hematoxylin (20–30 seconds) and the collagen fibers with Eosin, turning pink (2 minutes). For MT staining, we marked the nuclei with Hematoxylin (20–30 seconds) and the collagen fibers were marked with blue (30 seconds). Also, for mucopolysaccharides and glycosaminoglycans staining in the placental structure, we used the PAS technique, and we also marked the nuclei with Hematoxylin. For the immunohistochemical study, we marked the tissue edges with a hydrophobic marker (DakoPen), we performed the antigenic unmasking by boiling in the microwave oven (650 W, seven cycles × 3 minutes) in citrate solution (pH 6). We washed the slides with dH_2O (3×5 minutes), we inactivated the presence of endogenous peroxidase with 3% hydrogen peroxide solution (H_2O_2) for 30 minutes, we washed the slides again with dH_2O (2×5 minutes, to remove H_2O_2) and with 1% phosphate-buffered saline (PBS) (1×5 minutes), we blocked non-specific tissue sites using 3% skim milk (30 minutes). Then, the tissue sections were coated with the primary antibody (Table 1) and placed in the refrigerator, at 4°C (18 hours). The next day, the slides were allowed to warm to room temperature (30 minutes), washed in PBS (3×5 minutes) to remove the primary antibody, and then the secondary antibody was dripped (mouse VC002-025, R&D Systems, VisUCyte HRP Polymer) over them and allowed to bind to the primary antibody for one hour. The sections were then washed in PBS (3×5 minutes) and developed with 3,3'-Diaminobenzidine (DAB) (Dako). At the end, the nuclei were highlighted with Hematoxylin, the water was removed from the tissues by dehydration in ethanol baths with increasing concentrations [70%, 90%, 96%, and 100% (×5 minutes/each ethanol bath)], the sections were clarified in three xylene baths for 45 minutes (3×15 minutes/each bath) and small slides were applied with balsam of Canada.

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.

Results

Each pregnant patient, with a gestational age of more than 37 weeks, at term, who was introduced in the study did not present other conditions associated with pregnancy apart from those addressed in this study (fetus – IUGR, mother – TPh). All pregnant women were investigated from a clinical and imaging point of view, and the observation sheet was accompanied by their written consent or that of the legal representative in order to carry out this research based on the use of clinical data and macro-/microscopic photographs of tissue pieces (placentae). The age of the patients ranged from 17–45 years for mothers with TPh with a single fetus, with a mean age of 27.86 years (±6.75) and between 15–42 years for mothers with a single fetus who presented IUGR, with an average age equal to 28.2 years (±8.27) (Figure 1).

The pregnant women included in the study were weighed, and their weight varied as follows: for mothers with TPh with a single fetus, it varied between 55–92 kg, with an average of 73 kg (±10.09 kg), and for mothers with girls with IUGR was between 49–87 kg, with an average of 66.96 kg (±9.58) (Figure 2).

Regarding the gender of the newborns included in the study, we counted 16 female and 14 male newborns with mothers with TPh and 17 single girls with female IUGR and 13 males (Figure 3).

The weight of female newborns with mothers with TPh (F-TPh) varied between 2100–3100 g, with an average value equal to 2772.5 g (±263.19 g), and the weight of male newborns with mothers with TPh (M-TPh) varied between 2650–3860 g, with an average variance equal to 3259.28 g (±388.8 g), and the weight of female newborns with IUGR (F-IUGR) varied between 2310–2980 g, with an average value equal with 2691.35 g (±172.53 g), and the weight of male newborns with IUGR (M-IUGR) varied between 2100–3100 g, with an average value of 2630 g (±292.34 g) (Figure 4).

The placental weight of female newborns with mothers with TPh (F-TPh) ranged from 350–525 g, with an average value equal to 461.18 g (±45.73 g), and the placenta of male newborns with mothers with TPh (M-TPh) had a weight between 440–640 g, with an average value equal to 542.14 g (±64.36 g). The placental weight of female newborns with IUGR (F-IUGR) was between 385–496 g, with an average value equal to 448.05 g (±28.71 g), and the weight of male newborns with IUGR (M-IUGR) was between 396–516 g, with an average value equal to 439.3 g (±50.78 g) (Figure 5).

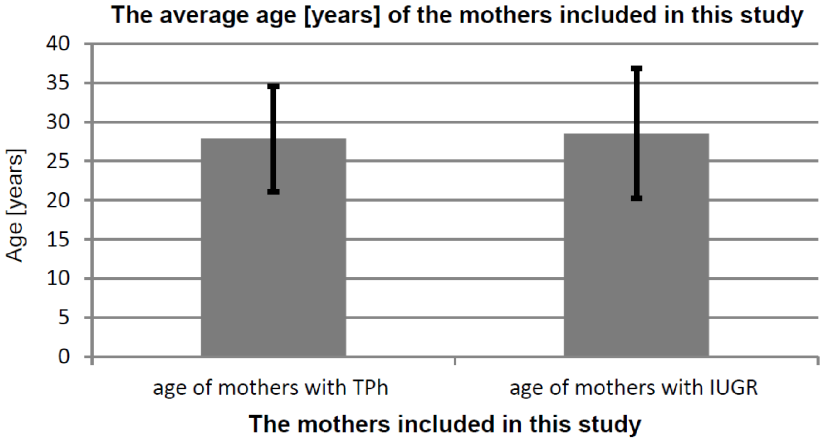

Figure 1 – ***The average age (years) of the mothers included in this study. Mothers average age was not significantly lower for the mothers with TPh compared to the mothers with IUGR, t(60)=-0.324, p=0.373. TPh: Thrombophilia; IUGR: Intrauterine growth restriction.***

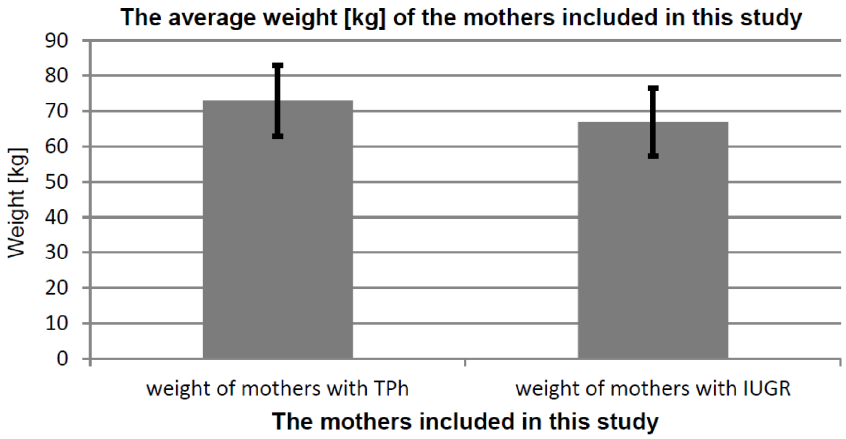

Figure 2 – ***The average weight (kg) of the mothers included in the study. Mothers average weight was not significantly lower for the mothers with TPh (73±10.09 kg) compared to the mothers with IUGR (66.96± 9.58 kg), t(60)=2.373, p<0.0104. TPh: Thrombophilia; IUGR: Intrauterine growth restriction.***

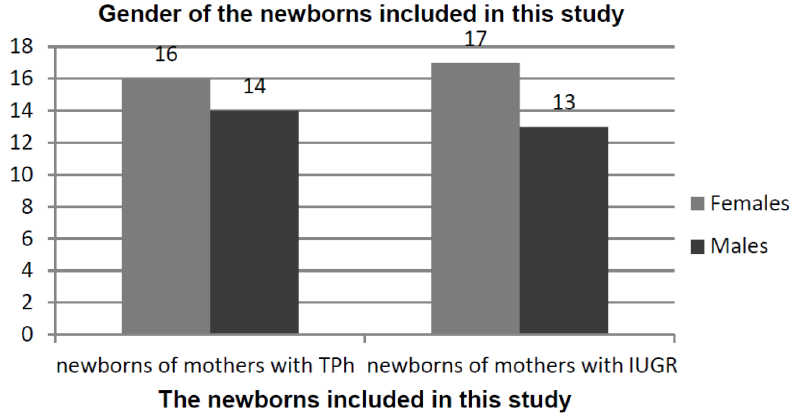

Figure 3 – ***Gender of newborns included in the study. The distribution of males/females was not significantly different for the newborns group of mothers with TPh and for the newborns group of mothers with IUGR, χ^2 (n=60)=0.4581, p=0.795. TPh: Thrombophilia; IUGR: Intrauterine growth restriction.***

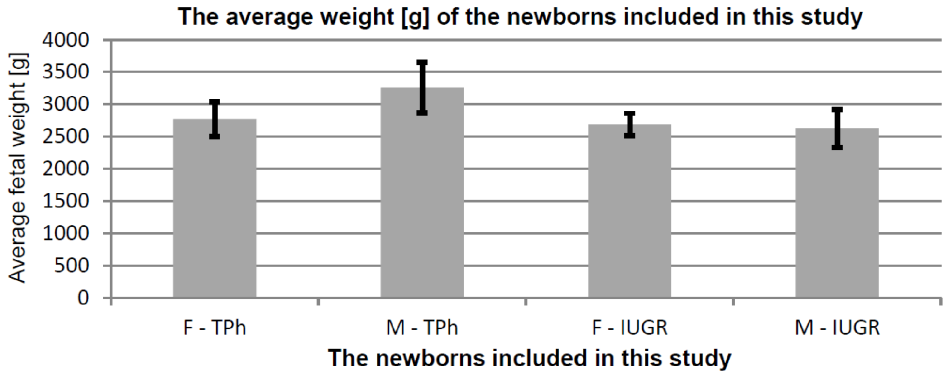

Figure 4 – ***Mean weight of fetuses according to the type of gestational pathology (TPh/IUGR) and to the newborn gender. There was a globally very significant difference between the weight of the newborns for these groups, F(3, 59)=14.413, p<0.001. F: Female; M: Male; TPh: Thrombophilia; IUGR: Intrauterine growth restriction.***

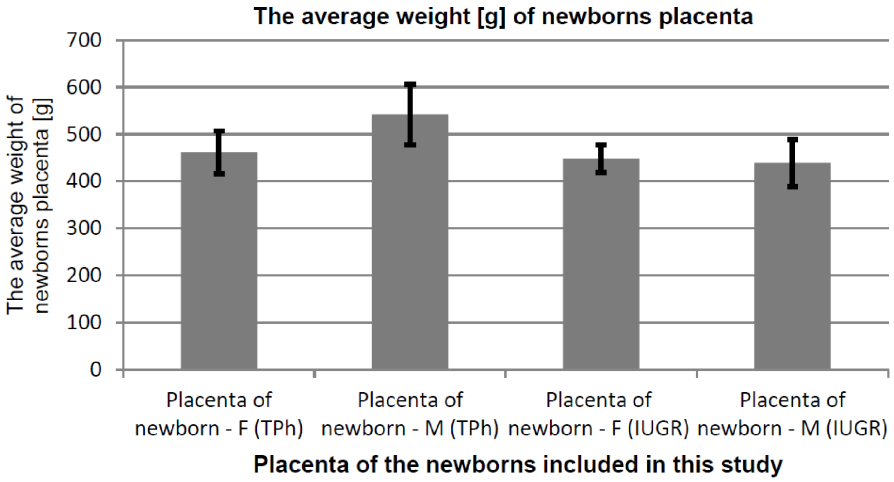

Figure 5 – ***Mean placental weight of fetuses introduced to the study, according to the type of gestational pathology (TPh/IUGR) and to the newborn gender. There was a globally very significant difference between the placental weight of the newborns for these groups, F(3, 59)=13.617, p<0.001. F: Female; M: Male; TPh: Thrombophilia; IUGR: Intrauterine growth restriction.***

Macroscopic aspects of placentae obtained from the two current study groups (mothers with TPh/newborns with IUGR), but also placentae from control groups (placentae of only newborns obtained from pregnancy not associated with any pathology, group discussed in a previous research [14]) underlined the characteristics of the two placental faces (maternal and fetal), the presence of amniotic membranes, the presence of the umbilical cord, but also the presence of certain morphopathological changes (Figures 6–9).

The classical microscopic study showed, through the classical HE, MT and PAS–Hematoxylin stainings, the normal placental villi but also their structural changes: intravillous and extravillous fibrinoid deposits, placental calcifications, thrombosis, and intravillous infarcts. The immunohistochemical study using the anti-cluster of differentiation 34 (CD34) antibody highlights the endothelial cells of the neoformation blood vessels and it was observed that the numerical density is much lower in the placentae associated with TPh and IUGR, compared to the normal placentae in the control group [16] (Figures 10–12).

Through the classical HE staining, we identified both the normal placental villous structure, with the collagen fibers stained in pink and the syncytiotrophoblast, but also the modified structural aspects: the calcification areas (stained in purple) and the areas with variable fibrinoid dimensions (stained in pink). Through the classical PAS–Hematoxylin staining, we highlighted the intravillous and extravillous vascular basement membranes and the fibrinoid deposits rich in glycosaminoglycans (pink-purple areas). Through the classical MT staining, we visualized the placental villi, the collagen fibers (stained in blue), the calcification areas (stained in red purple), and the intravillous and perivillous fibrinoid areas (stained in red). Through the immunohistochemical staining with the anti-CD34 antibody, we are staining the intravillous and intervillous vascular endothelium (brown staining), and we noticed that their number is much smaller when the placenta was associated with TPh or IUGR, compared to the placental vascular density obtained from single pregnancy without associated pathologies (control group) [16].

For the statistical study of vascular density, we made four images with ×200 lens from the same placental section of each case (30 cases of placenta associated with TPh and 30 cases of placenta associated with IUGR), sections with immunohistochemically staining, then we counted intravillous and perivillous placental small blood vessels of each case and we performed a comparative statistical study using the Microsoft XL program. We observed that vascular density is much lower in both placentae associated with TPh and in those associated with IUGR, compared to the control group in the previous study [16]. For the placenta associated with TPh, the vascular density varied between 34–71 vessels/×200, with an average value of 53.16 vessels/×200 (±8.53 vessels/×200), and for the placenta associated with IUGR, the vascular density varied between 27–75.25 vessels/×200, with an average value of 54.82 vessels/×200 (±9.66 vessels/×200) (Figure 13).

We also made a comparison between maternal weight

and age and noticed that they can increase in direct proportion (Figure 14; Table 2). Comparing fetal weight and placental weight, we also noticed an increase directly proportional between them (Figure 15; Table 3).

And in the last part of this study, we compared the placental vascular density with the placental weight, and we noticed that there is an inversely proportional relationship between them (Figure 16; Table 4).

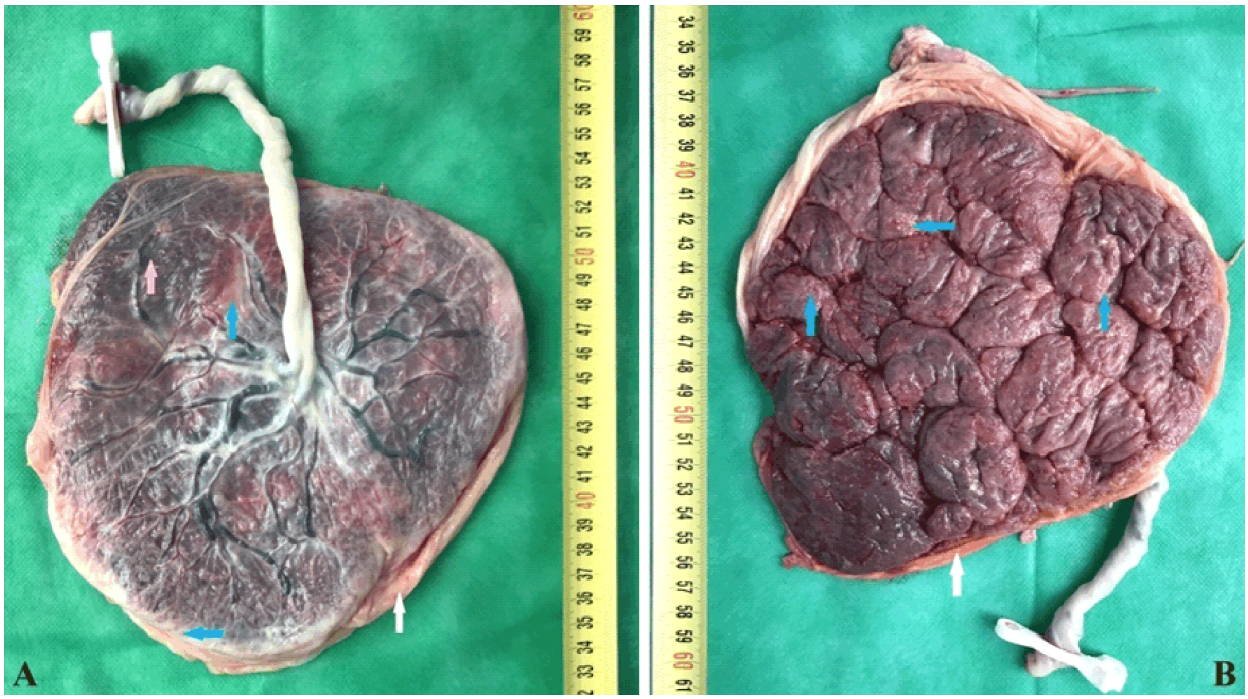

Figure 6 – ***Normal aspects of the placenta at term from the single pregnancy not associated with any pathology – control group [16]: (A) Fetal placental face – amniotic membrane (white arrow), amniotic cord inserted centrally, small fibroid deposits that may occur in the mature placenta, term pregnancy (blue arrows) and small calcifications (pink arrow); (B) Placental maternal side – amniotic membrane is observed at the periphery (white arrow) and normal placental villi, with small fibrinoid deposits (blue arrows). Images from the collection of Dr Anca-Maria Istrate-Ofiţeru.***

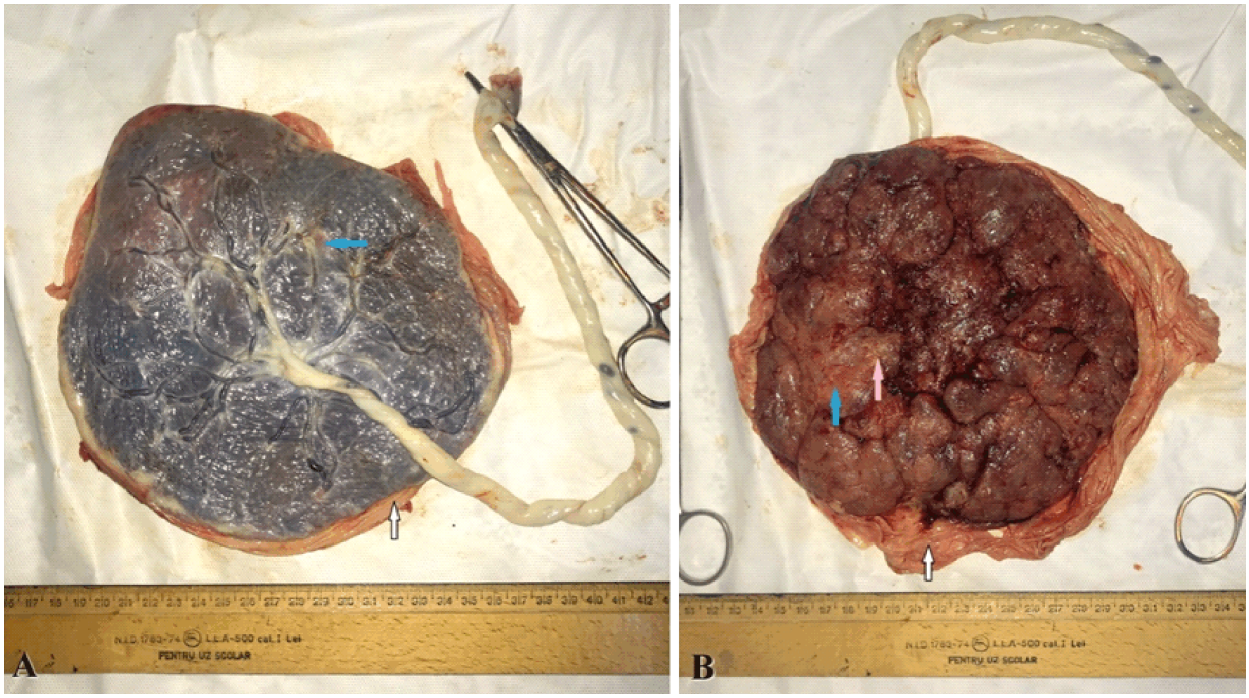

Figure 7 – ***Aspects of the placenta at term from the single pregnancy associated with TPh: (A) The placental fetal face – the amniotic membrane (white arrow) is observed, the amniotic cord inserted centrally, large fibrinoid deposits that modify the placental architecture (blue arrow); (B) Placental maternal side – the amniotic membrane at the periphery is observed (white arrow) and the placental villi modified by the presence of fibrinoid deposits (blue arrow) and calcifications (pink arrow). TPh: Thrombophilia. Images from the collection of Dr Anca-Maria Istrate-Ofiţeru.***

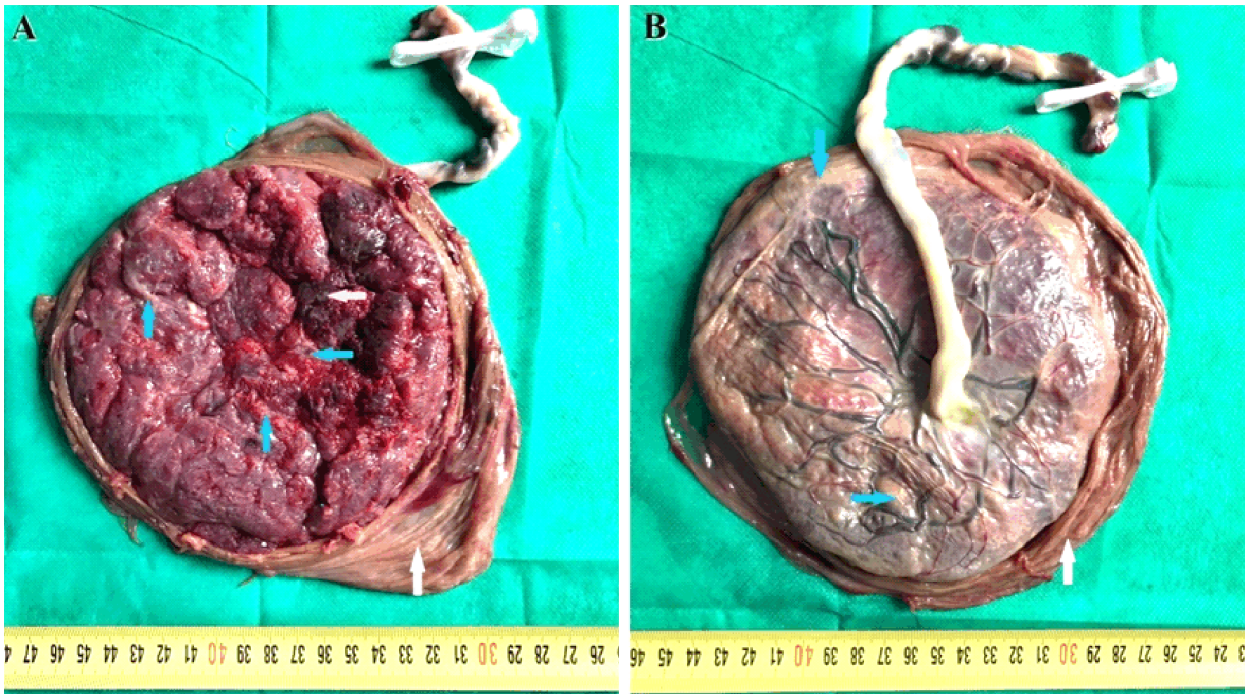

Figure 8 – ***Aspects of the term placenta from single pregnancy with fetus with IUGR. The small placental size is observed: (A) Placental maternal side – identify the amniotic membrane located at the periphery (white arrow), placental villi modified by the presence of fibroid deposits (blue arrows) and calcifications (pink arrow); (B) The placental fetal face – the amniotic membrane at the periphery (white arrow) and the placental villi modified by the presence of fibroid deposits (blue arrow) are identified. IUGR: Intrauterine growth restriction. Images from the collection of Dr Anca-Maria Istrate-Ofiţeru.***

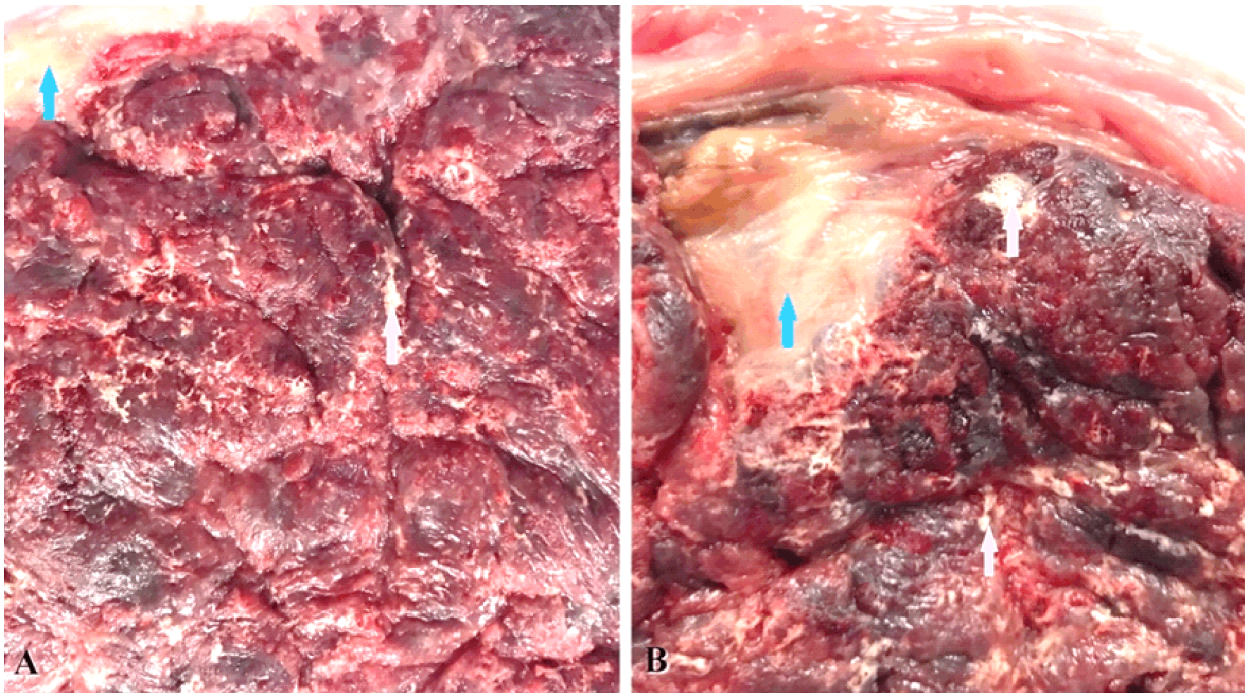

Figure 9 – ***Aspects of the term placenta from single pregnancy with fetus with IUGR: (A and B) The placental maternal face is identified that presents placental villi modified by the presence of fibrinoid deposits (blue arrows) and calcifications (pink arrows). IUGR: Intrauterine growth restriction. Images from the collection of Dr Anca-Maria Istrate-Ofiţeru.***

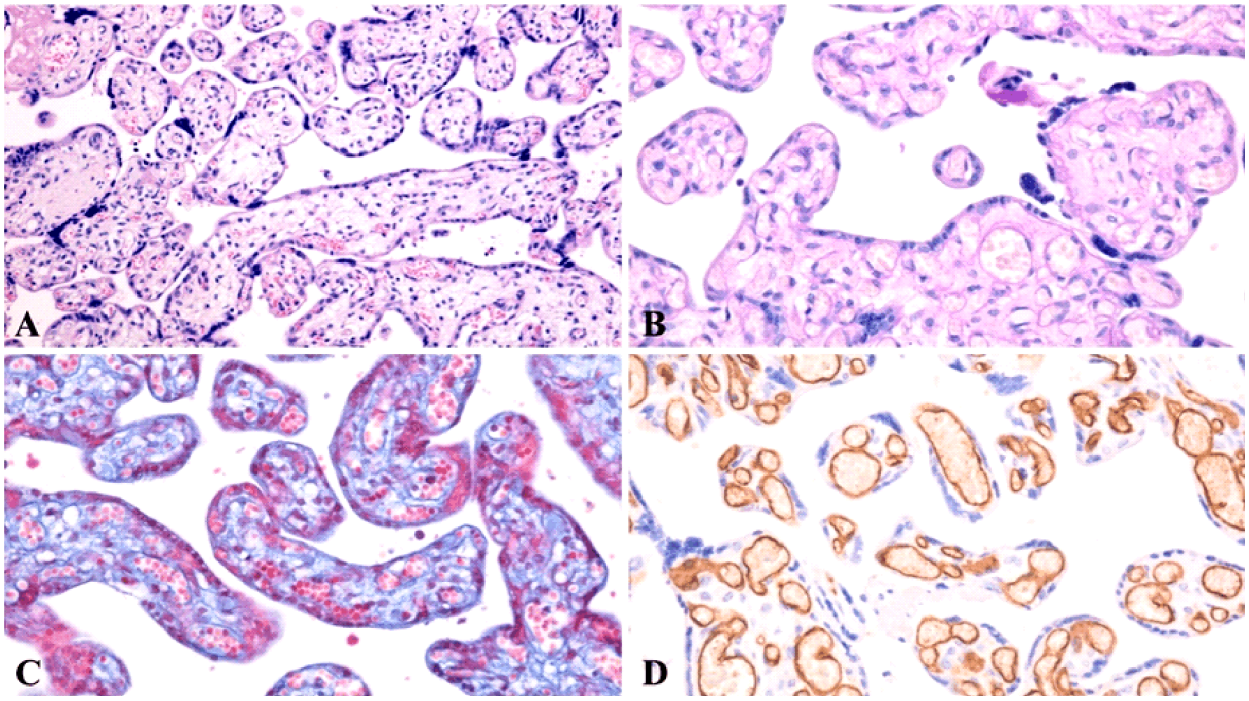

Figure 10 – ***Placental microscopic aspects of single pregnancy – control group [16]: (A) Normal placental villi (HE staining, ×200); (B) Normal placental villi at which the vascular basement membranes are highlighted with PAS–Hematoxylin and small areas of fibrinoid deposition (pink-purple colored) are identified (×200); (C) Normal placental villi with small deposits of perivillous and intravillous fibrinoid (red colored) (MT staining, ×200); (D) The endothelial layer of the small, intravillous blood vessels was highlighted in brown (Anti-CD34 antibody immunostaining, ×200). 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-Ofiţeru.***

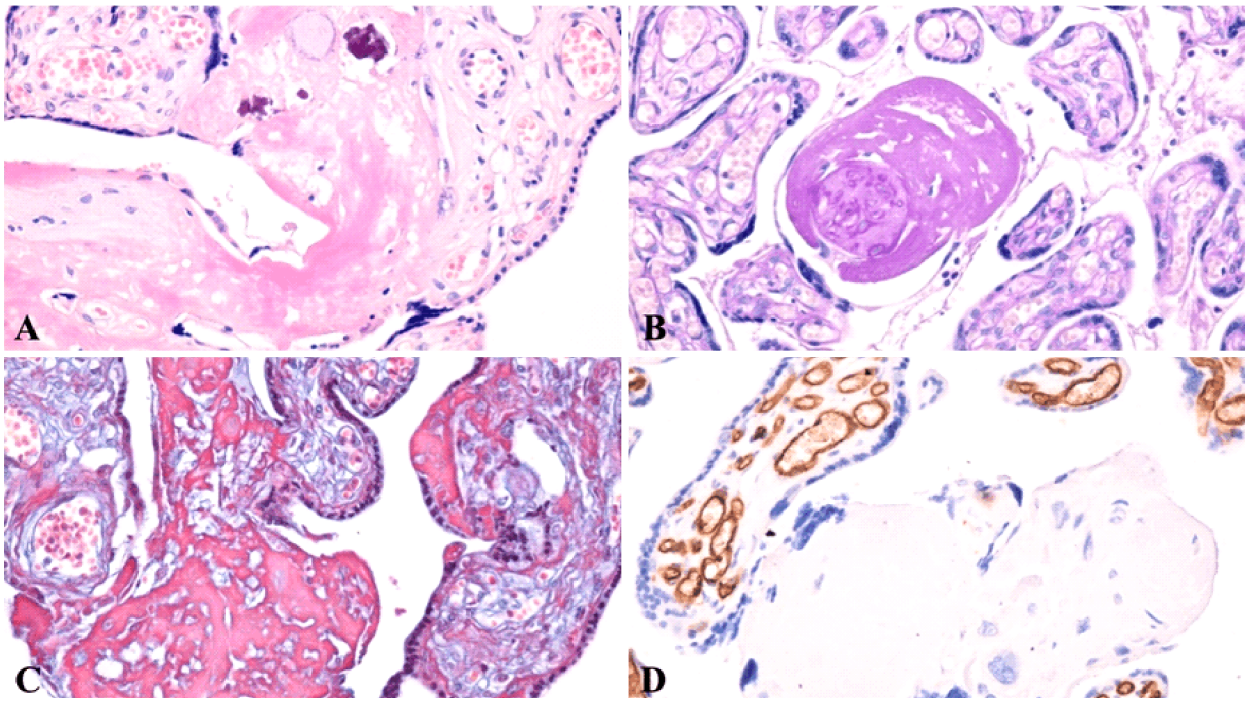

Figure 11 – ***Placental microscopic aspects of pregnancy associated with TPh: (A) Heavily modified placental villi, with areas of calcification (purple areas) at the villous periphery, with large areas of placental infarction and deposition of perivillous and intravillous fibrin (HE staining, ×200); (B) Intensely modified placental villi, at which the vascular basement membranes are highlighted with PAS–Hematoxylin, but also the large areas of fibrinoid caused by villous infarction (pink-purple colored) (×200); (C) Placental villi with perivillous and intravillous massive deposition of fibrinoid (red colored) (MT staining, ×200); (D) The vascular endothelium of the intravillous vessels was highlighted in brown (Anti-CD34 antibody immunostaining, ×200). 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-Ofiţeru.***

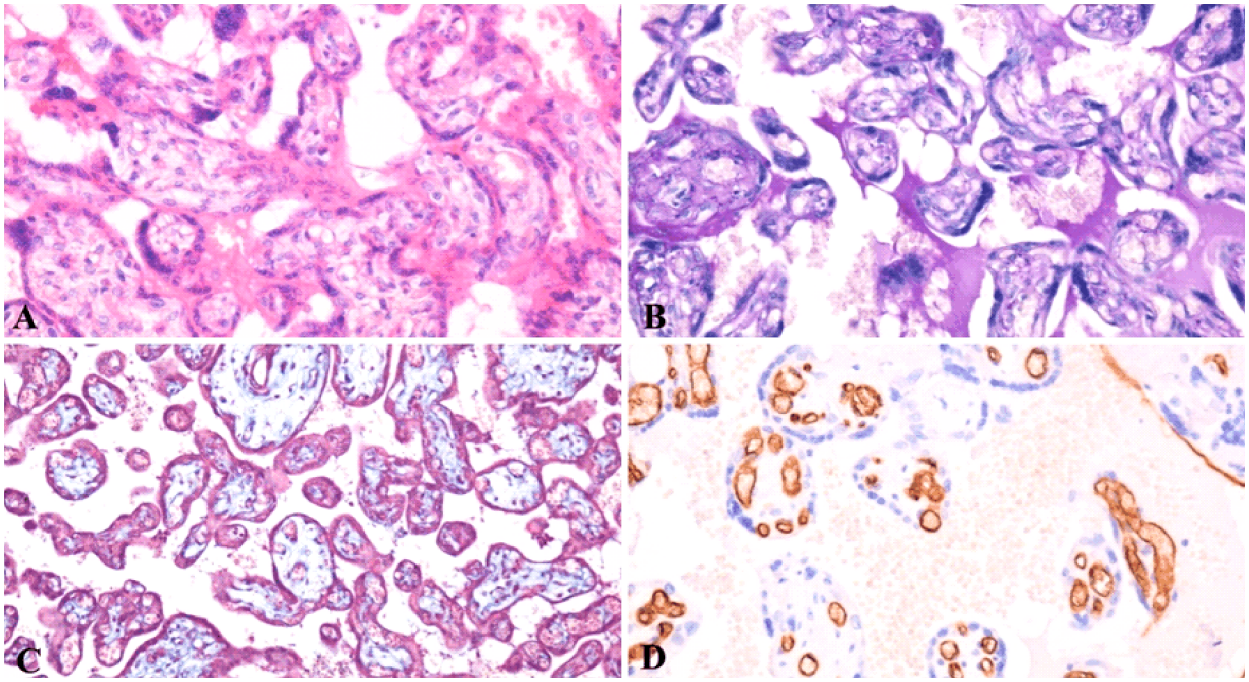

Figure 12 – ***Placental microscopic aspects of pregnancy associated with IUGR: (A) Strongly modified placental villi, hypovascular, with calcification areas (purple areas) at the microvilli periphery, with extensive areas of perivillous and intravillous fibrinoid deposition with a "canvas" appearance (HE staining, ×200); (B) Intensely modified placental villi, at which the vascular basement membranes are highlighted with PAS–Hematoxylin, but also the large areas of intra-/perivillous fibrinoid with the appearance of a "canvas" (pink-purple colored) (×200); (C) Placental villi with perivillous and intravillous fibrinoid deposition, with "canvas" appearance (red colored) (MT staining, ×200); (D) The vascular endothelium of the small intravillous vessels, reduced in number, was highlighted in brown (Anti-CD34 antibody immunostaining, ×200). 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-Ofiţeru.***

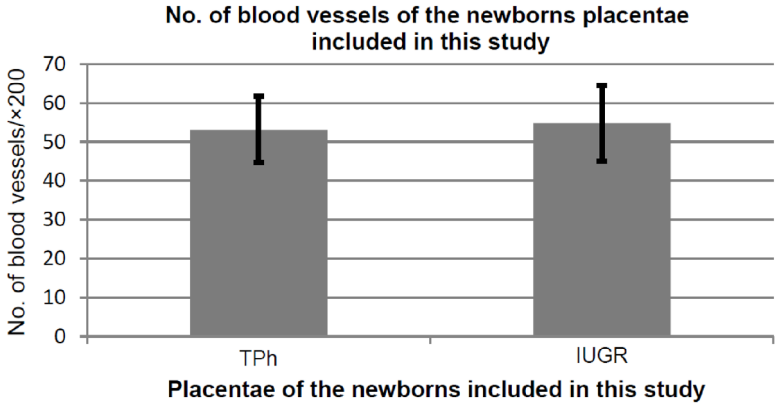

Figure 13 – ***Mean vascular density from placentae of mothers with TPh and IUGR. There was not a globally very significant difference between the number of blood vessels/×200 of the newborns placentae for these groups, F(1, 59)=0.496, p=0.483. TPh: Thrombophilia; IUGR: Intrauterine growth restriction.***

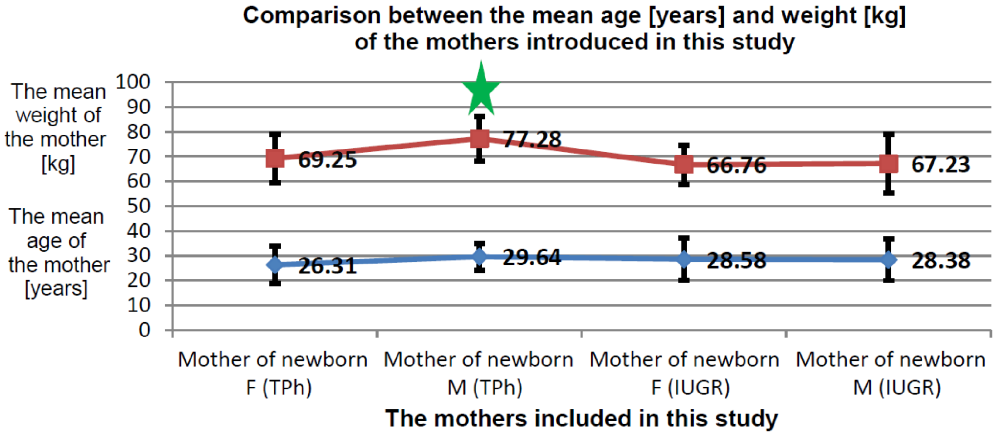

Figure 14 – ***Comparison between the mean age of the mothers according to the type of gestational pathology (TPh/IUGR), gender of the newborns and maternal weight. It is observed that there is a directly proportional relationship between their values (green star). F: Female; M: Male; TPh: Thrombophilia; IUGR: Intrauterine growth restriction.***

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

	Mother of newborn – F (TPh)	Mother of newborn – M (TPh)	Mother of newborn – F (IUGR)	Mother of newborn – M (IUGR)
t Stat	-13.880	-17.068	-13.677	-9.647
P(T<=t) one-tail	*p*<0.005	*p*<0.005	*p*<0.005	*p*<0.005

F: Female; M: Male; TPh: Thrombophilia; IUGR: Intrauterine growth restriction.

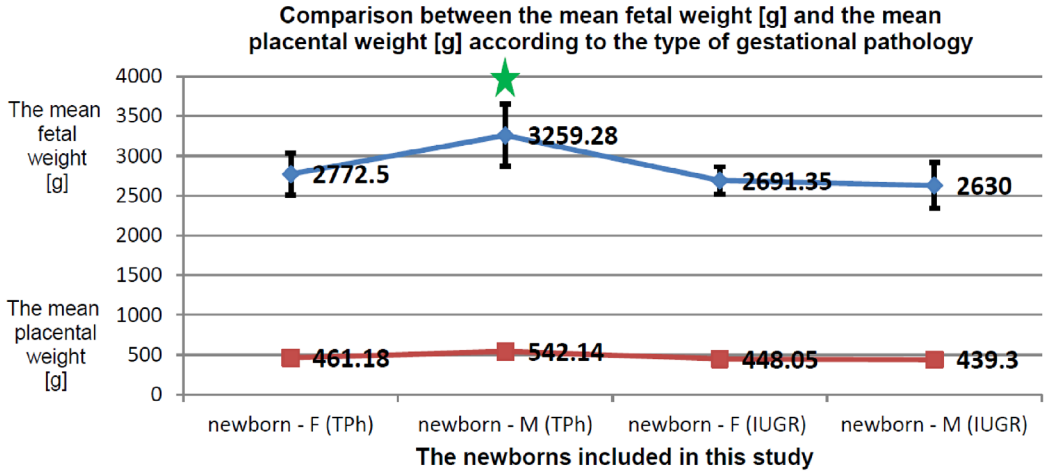

Figure 15 – ***Comparison between the mean fetal weight and the mean placental weight according to the type of gestational pathology (TPh/IUGR). We noticed that the fetal weight values increase directly proportional to the placental weight values (green star). F: Female; M: Male; TPh: Thrombophilia; IUGR: Intrauterine growth restriction.***

Table 3 – ***Comparison between the mean fetal weight and the mean placental weight of the newborns according to the type of pregnancy***

	Newborn – F (TPh)	Newborn – M (TPh)	Newborn – F (IUGR)	Newborn – M (IUGR)
t Stat	34.608	25.796	52.881	26.619
P(T<=t) one-tail	*p*<0.005	*p*<0.005	*p*<0.005	*p*<0.005

F: Female; M: Male; TPh: Thrombophilia; IUGR: Intrauterine growth restriction.

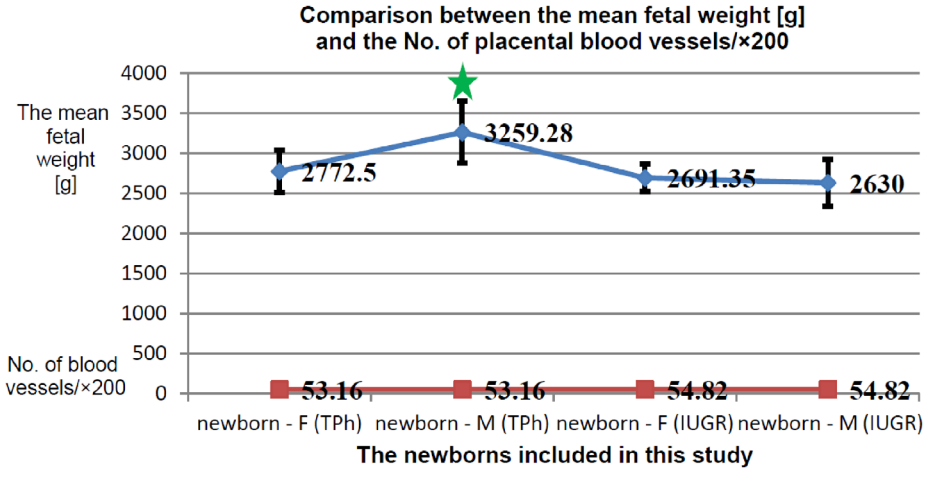

Figure 16 – ***Comparison between mean placental vascular density and fetal weight. We noticed that the fetal weight values do not increase directly proportional to the placental weight (green star). F: Female; M: Male; TPh: Thrombophilia; IUGR: Intrauterine growth restriction.***

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

	Newborn – F (TPh)	Newborn – M (TPh)	Newborn – F (IUGR)	Newborn – M (IUGR)
t Stat	57.104	45.767	84.18	48.97
P(T<=t) one-tail	*p*<0.005	*p*<0.005	*p*<0.005	*p*<0.005

F: Female; M: Male; TPh: Thrombophilia; IUGR: Intrauterine growth restriction.

Discussions

The placenta is an organ with many roles in the stages of fetal development, and its aspects may vary depending on the type of pregnancy, single or multiple, the type of placenta, fused or not, depending on the associated pathologies, but all this can be dictated by maternal clinical aspects (age, weight) [16–22]. In this study we observed that there is a directly proportional relationship between maternal weight and age, between the type of associated pathology (TPh or IUGR), child gender and maternal weight, between fetal weight and placental weight and an inversely proportional relationship between fetal weight and the number of capillaries with immunohistochemically staining.

The diagnostic strategies of TPh and IUGR are absolutely necessary because obstetrical management can prevent negative neonatal evolution, mortality and morbidity being constantly increasing. Therefore, morphological and morphopathological evaluation of the placenta is a common practice, and changes present in both TPh and IUGR are frequently addressed in the literature [23, 24]. In these pathologies, vascular placental changes are frequently encountered that result in fetal malperfusion, but there are many studies that claim by Doppler analysis that there is fetal vascular redistribution [25].

Besides the clinical aspects approached, the other objectives of this study were to highlight the placental morphopathological aspects and to observe if there is any special feature in these two associated pathologies. We observed that the placentae were smaller than the normal groups [16], and the fetal weight varied in direct proportion to the placental weight. Macroscopically, we have identified aspects that may be present in other pathologies, fibrinoid deposits and calcifications. Microscopically, we identified vast aspects, which were found in different percentages in other pathologies associated with pregnancy [23–26].

In the evolution of pregnancy, the placenta has several stages of development and maturation, adapting to fetal requirements and gestational development [25], and in terms of IUGR, the etiology can have several approaches: maternal, fetal or placental [26–28]. The placental cause can be represented by the contact area with the uterine wall, the place where the spiral arteries are found, which in this case shows a remodeling and no longer ensures the normal placental flow [29, 30]. Placental vascular change leads to decreased maternal flow to the growing fetus. There are not many studies that accurately describe the placental changes associated with IUGR, but through our research, we highlighted a few aspects. We noticed that there may be a deficiency of microvascularization, making a comparison with the placenta in single or multiple pregnancy unassociated with different pathologies, aspects demonstrated by quantifying the placental vascular density by immunolabeling with anti-CD34 antibody [16–22]. Some studies have shown that there may be both global and partial placental malformation, associated with increased maturation of placental villi or chronic villi, accompanied by decreased segmental fetal vascularization (fetal thrombotic vasculopathy) and vascular and stromal lesions [31].

Carrying out a review of the types of placental pathologies in pregnancies associated with TPh or IUGR, but also with gestational diabetes or gestational hypertension, we observed that the most common histopathological features were chorangiosis – large number of microvessels in placental villi (39%), placental ischemia and placental infarction (18%), chronic villi with unidentified etiology (13%), fetal thrombotic vasculopathy (8%) and to a lesser extent veil changes in umbilical cord insertion, accreted placenta [32, 33]. Similar changes have been described for complicated pregnancies with placental insufficiency and IUGR, gestational hypertension or spontaneous idiopathic labor [24, 34, 35].

We identified a number of placental histopathological changes associated with TPh and IUGR: thrombosis, calcification, infarction, massive intravillous or extravillous fibrinoid deposits, sometimes with "canvas" aspects, which may occur under the influence of immunogenic factors altering vasculogenesis and placental quality [36]. Depending on the size of the lesions and the time of their occurrence during pregnancy, IUGR may be associated as a consequence [37, 38], and the link between suppression of vascularization by infarction and fetal hypoxia have already been described in the literature [39].

Redline [31] showed that fibrinoid deposits may be associated with influencing placental weight and thus fetal weight, which was demonstrated in our study, where we observed that placentae and girls with IUGR were significantly lower compared to placentae and fetuses from single normal pregnancies [16, 31]. Diffuse calcification has been found frequently in the placenta at term, matures and demonstrates its senescence and effects on pregnancy [40]. İskender-Mazman *et al.* [40] did not find a correlation between IUGR and diffuse placental calcification [41], but other studies have shown the presence of syncytial nodes as the cause of placental ischemia [42].

Also, the presence of avascular villi, found in areas of placental infarction, have been identified more frequently in placentae during pregnancy with restricted fetuses [43, 44]. We understand that angiogenesis is a fundamental element in vascular expansion and the appearance of terminal villi and through its failure IUGR can occur [45].

Placental vascular development begins in early pregnancy and continues during pregnancy [46]. Vascular endothelium of neoangiogenesis vessels has been widely evaluated by means of immunohistochemistry techniques [47], so by using anti-CD34 antibody, vessels from mature stem and intermediate villi were labeled, in contrast to research by Mackiewicz *et al.* [47] who identified the presence of immunoreactivity in the small capillary endothelium of IUGR-associated placentae [48]. In our study we observed the highlight of the endothelium of small, capillary vessels in the terminal villi.

The placenta of patients with TPh can present many lesions that appear gradually during pregnancy, which can have repercussions on the fetus, as we mentioned, through vascular dysfunction, the consequence of ischemic lesions [49]. Also, severe ischemia can occur due to the presence of multiple vascular thrombosis in the placental structure, due to the hypercoagulant status of the patient. Beeksma *et al.* [49], showed in a study of placentae harvested from patients with newborns with IUGR that there is no link between placental changes and TPh and suggested that other factors may be the cause of placental pathology [49].

In our study, through the classical histological staining techniques we identified most of the placental lesions described in the literature, and as a peculiarity, we noticed the presence of perivillous fibrin deposits in the structure of the placentae from newborns with IUGR, appearance of particular deposits, of "textile" or of "canvas".

Conclusions

Through this study we were able to realize and demonstrate that there is a direct proportional link between maternal and fetal clinical aspects, the mother's age can increase with her weight and fetal weight. Also, the placental weight is directly proportional to the fetal weight, but the number of small blood vessels with immunohistochemical staining, are inversely proportional to the placental weight. Through the microscopy techniques, we identified a series of placental pathological aspects, present in both TPh and IUGR. These changes are represented by fibrin deposits, calcifications, vascular thrombosis, placental infarction and are associated with consequences on fetal growth and development.

Conflict of interests

The authors declare that they have no conflict of interests.

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Authors' contribution

Nicoleta-Loredana Voicu and Roxana Elena Bohîlţea equally contributed to this article.

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