The macroscopic examination of the placental vasculature with a corrosive agent

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Abstract
The placental development is a complex process, in which any disturbance can induce changes with grave consequences for the offspring. Villi and villous vascularization development is in, and underdevelopment can lead to villous placental insufficiency. This paper aims to analyze the arterial and venous vasculature of the placenta, both macroscopic and morphologically, by different injection techniques of contrast agents (plastic substances type AGO II) in 50 human placentas with different ages of gestation.

Keywords: arterial and venous vasculature, corrosion concoction, maternal placenta, fetal placenta.

Introduction
The placenta – the organ with a particular vascular structure, of which functional integrity is dependable the birth of a new life – was the subject of numerous studies over time. The concept of placental circulation dates back to antiquity. At the end of the first century, Soranus described the chorion, the amnion and the cord containing four pots. For many years, until the 16th century, the maternal and fetal circulations were considered continuous.

In the years that followed, the arguments concerning the pure fetal or maternal–fetal origin continued, as well as the ones on the momentum of the connection between the two systems. The question posed by Ramsey – when exactly is established the real uteroplacental circulation? – or the one about how maternal blood enters the intervillous space, as well as the theories issued by Borell, Panigel or Ramsey, are still debated today.

The normal development of the placenta is a major factor in the fetus’s growth and development, whilst the maternal-fetal placental vasculature is essential in this regard. During placentation, there is an ongoing process, which combines angiogenesis with vasculogenesis, as demonstrated by numerous studies, which reveal important roles of various known angiogenic factors, while other studies show the roles of different classes of factors in vascular morphogenesis nonspecific to the placenta.

Placentation includes extensive angiogenesis in maternal and fetal placental tissues, accompanied by a marked increase in uterine and umbilical blood flows [1–6].

Materials and Methods
We studied 50 human placentas collected immediately after naturally performed births in the “Bega” Clinic of Obstetrics and Gynecology, Emergency County Hospital, Timișoara, Romania, from 2012 to 2013. After the macroscopic evaluation and weighing of the placentas, they were processed in the Laboratory of Anatomy and Embryology, “Victor Babeș” University of Medicine and Pharmacy, Timișoara.

The examination of the placentas was made soon after delivery, after a protocol of a quick description of the placenta and the associated structures. Then, the placentas were transported to the Laboratory of Anatomy and Embryology, “Victor Babeș” University of Medicine and Pharmacy, Timișoara, to obtain the corrosion concoction.

Study of the corrosion concoction
The method used in the case of our study is a standardized method of the Laboratory of Anatomy and Embryology, “Victor Babeș” University of Medicine and Pharmacy, Timișoara, for over 50 years. The making of corrosion concoction followed the recipe of the laboratory using type AGO II plastic substances, differently colored and then subjected to the corrosive action of hydrochloric acid. This technique has been published and reported in the national and international anatomical press, under the signature of Prof. Gheorghe Corondan and Lecturer Leonida Bejan in 1956, and was later improved by Ciobanu (1960), Diaconescu (1962), Radu (1968), Kuhn (1961), Rottenberg (1969). This technique produces corrosive agents, which are the mold vessels in the organ studied – in our case – the placenta. These preparates provide a 3D image that is very close to real, thus allowing the study of vascular arborization.

Macroscopic, we analyzed the branching ways of the allantochorionic vessels from the umbilical vessels, after
which their shooting were next. Then, we schematized the preparations to get a clearer picture of the allanto-chorionic vessels. Macroscopic vascular morphometry was used in the determination of vascular parameters: length, diameter. For this, we used rulers, graded compasses, graded bands and micro-callipers.

Results

The normal development of the placenta remains a major factor in the growth and development of the fetus, and the maternal–fetal placental vasculature is essential in this regard.

Placental angiogenesis has a different local component of angiogenesis found in other anatomical regions.

Placental development is a complex process; the villous vasculature is in, and its dysfunction could lead to placental insufficiency.

The age of the patients ranged from 19 to 36 years, 17 of which were primiparous.

Gestational age of the pregnancy ranged between 32–40 weeks (Table 1, Figure 1).

Table 1 – Gestational age of pregnancy and number of placentas

<table>
<thead>
<tr>
<th>Gestational age of pregnancy [weeks]</th>
<th>No. of placentas</th>
</tr>
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<tbody>
<tr>
<td>32</td>
<td>5</td>
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<tr>
<td>33</td>
<td>3</td>
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<td>34</td>
<td>8</td>
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<td>38</td>
<td>5</td>
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<tr>
<td>39</td>
<td>9</td>
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<tr>
<td>40</td>
<td>4</td>
</tr>
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</table>

Figure 1 – Gestational age of the studied placentas.

The weight of the placentas ranges between 320–480 g, as shown in Table 2 and Figure 2.

After examining the placental surfaces, we went on to counting the cotyledons. Thus, on average, we obtained a total of 28 cotyledons (average value).

Table 2 – Weight of studied placentas

<table>
<thead>
<tr>
<th>Placental weight [g]</th>
<th>No. of placentas</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;350</td>
<td>7</td>
</tr>
<tr>
<td>350–400</td>
<td>12</td>
</tr>
<tr>
<td>400–450</td>
<td>17</td>
</tr>
<tr>
<td>450–500</td>
<td>14</td>
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</tbody>
</table>

Figure 2 – Weight of the studied placentas.

The thickness of the placenta was between 1.4 cm and 3.7 cm; most cases had an average value of 2.4–2.6 cm (33 cases) (Figure 3).

Figure 3 – Thickness of the studied placentas.

Our study shows that the placental thickness increases until the second quarter of pregnancy, after which it remains constant, placental growth happening through the increase of diameters.

The form of the clinically examined placentas after delivery was variable, most having discoidal form.

From a macroscopic viewpoint, the placenta has a maternal face and a fetal face.

The maternal face: consists of basal obsolete. On it, there is a series of grooves delimiting placental lobes. The grooves correspond to the intercotyledonary septa.

In five cases diagnosed with fetal and placental hypotrophy, on the maternal face we had noticed stronger regions, probably fibrin deposition or old infarctions.

The fetal face: it is smooth, glossy, translucent, allowing visualization of the vascular design. It is covered by amnion and by the deeper part of the chorionic plate, beneath which are the umbilical vessels (Figures 4–6).

In humans, the placenta has the shape of an umbrella, and at the periphery of the placenta, the intervillous spaces communicate with a smaller circular canal of small dimensions, called the marginal sinus. The villi development and cotyledons formation consist of the separation from the villous plate of around 15–30 big first order villous trunks. Of these, at a short distance from the chorionic plate, the second order villous trunks are detaching, and in turn, they give other branches, perpendicular on the basal plate, called third order villi (Figures 7–11).
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Discussion

The formation of the placenta starts in the first weeks of pregnancy, 8–10 days after the implantation of the egg in the uterine lining, and reaches maturity by the end of the third month of pregnancy.

Central, on the fetal face, there is the insertion of the umbilical cord. It is 40–70 cm long, 1–2 cm thick, of elastic consistency and has bluish-pink coloration. In its structure, it includes two arteries spirally arranged around a vein and surrounded by Warthon gelatin (American Pregnancy Association, January 2004) [7–10].

The ensemble of free villi and the ones derived from a first order villous trunk, form a cotyledon – the placental cotyledon represents the vascular unit of the placenta. The big cotyledons in the periphery of the
placenta are formed out of one single drum, as opposed to centrally located larger cotyledons, formed out of 2–5 drum systems. The assembly of several cotyledons forms a placental lobe – which is the functional unit of the placenta [11–14].

Vascular placental morphometry shows that the development of the vascular trunk, although impressive, contributes to a lesser extent in the placenta development, than the stroma; the vascular trunk develops the most in the second trimester of pregnancy.

The corrosive concoctions provide a three-dimensional study model of the placental vascularization, and it is relatively easy to perform, and easy to interpret. They allow the examination of the vascular trunk to the level of the second order villous vessels. This method represents the ideal model for the study on the vascularization of parenchymal organs – in our case, the placenta.

Regarding placental weight – shown in Table 2 and Figure 2 – we obtained a rise that indicated that the placental weight represents about one-fifth the weight of the fetus, not sixth, as in some studies in the literature [15–17]. Therefore, the term placentas present large weight variations. In the third trimester of pregnancy, the placenta grows more than the fetus. The placenta development is directly dependent on blood flow through the umbilical artery. Some of these variations are of genetic nature, because there are some differences between the genes that regulate the fetal growth, and the ones that regulate the growth of the placenta. Other factors that may interfere with the placental development include: storing blood in the placenta and in the umbilical cord, the length of the umbilical cord or the presence of membranes attached to the placenta. The blood in the umbilical cord and cotyledon drains in about 2–3 hours after delivery, reason for which we have conducted the correct determination of the placental weight, the second day.

The examination of the placental parameters – shape, weight, thickness – is important as a preliminary stage for the study of the placental architecture.

The examination of the placental surfaces provided data on the possible placental pathologies. We have not demonstrated the presence of shape placental abnormalities in our study.

Other factors that can lead to placental hypotrophy are accelerated maturation (consequence of pre-eclampsia, eclampsia, and maternal hypertension) and most fetal malformations. The association between low weight of the fetus and placental hypotrophy has as an explanation the inadequate perfusion of the fetus due to a small placenta with small vascular exchange surface. Smoking during pregnancy causes fetal hypotrophy, but it is not directly proportional to the placental retardation.

We carried out the counting of both villous vessels and tertiary villous, using for processing the data the placental vasculature microdensity – on a 400× field, defined as the number of vessels on a microscopic field and the villous vascular micro density, defined as the ratio of the number of vessels and the number of tertiary villous [18–24].

The vascular microdensity increases gradually as the pregnancy evolves, due to the branching of villous vessels, reaching a 23 vessels/field in placentas with a 32 week – diagnose of pregnancy, and a 49 vessels/field in placentas with a 39 week – diagnose of pregnancy.

We have thus seen a steady increase of the vascular micro density during the evolution of pregnancy. In this last trimester of pregnancy, we were able to observe the emergence of small tertiary villi, with a rich stroma, due to relative growth, but also due to vascular senescence processes.

The examination of the corrosive agents resulted in highlighting several aspects of the distribution of the allantochorionic vessels:

- at the level of venous territory we have not found any anastomosis;
- first order ramifications give second order ramifications, which give third order ramifications;
- third order ramifications penetrate the chorionic plate, giving first order villous branches;
- venous branches are then arranged in a pattern of dichotomous division or bus type (out of which much smaller diameter branches start);
- the arteries follow the path of the veins, but they do not always divide as such;
- at the level of arterial territory, we have noticed numerous anastomosis; we have met the most numerous anastomosis at the insertion site of the umbilical cord (in 43 out of 50 cases, which represents 86%).

Conclusions

The model of corrosive agents showed the known distributions as dichotomous distribution and bus type distribution. The dichotomous distribution is specific to central insertion, where there is a reduction of the vascular caliber, while the bus type distribution is characterized by the appearance of lateral insertion in peripheral areas, where the umbilical artery is responsible for ensuring the vascular perfusion. The normal placental development remains a major factor in the growth and development of the fetus, and the maternal–fetal placental vasculature is essential in this regard. Placental angiogenesis has a different local component of angiogenesis, found in other anatomical regions. The placental development is a complex process; the villous vasculature is in, and its dysfunction may lead to a placental insufficiency.

References

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