Correlations Between Chorial Villositary Vasculogenesis and Ultrasound Morpho-Functional Markers of The Trofoblast In The Normal Pregnancy

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ABSTRACT

Introduction. The placental vasculogenesis process appears only in the time of the embryo development and forms the primordial vascular substratum, the first capillary vessels developing in the days 18 – 20 from the fecundation, phenomena that are necessary for the normal evolution of the pregnancy. At the same time, at the level of the implantation site, the ultrasonographic investigation of the trofoblast has shown specific changes correlated with the gestational age.

Material and method. We have investigated 47 pregnant women with the gestational age between 5 and 12 weeks, which had legal abortion on request. The patients were echographically monitored until the time of the curettage, particularly by the trofoblastic thickness at the implantation site. The material resulted after the curettage has been histologically studied, the histological and echographical evaluation of the cases being made according to the gestational age.

Results and discussions. It has been accomplished a gestational histological grading by establishing some specific elements of the villositary development related to the gestational age, that has been correlated with the dates from the ultrasonographical evaluation. Conclusions. The trofoblastic vasculogenesis undergoes stadal phenomena specific to the first trimester chronology and the trofoblastic thickness at the implantation site level may be correlated with the gestational age. The correlations between those histologic and imagistic characteristics of the placenta at the implantation site may allow a better understanding of the phenomena that have their roots at this level, and it is necessary to have additional studies for determining if the changes of this phenomena are associated with the spontaneous abortion.

KEY WORDS vasculogenesis; trofoblast; ultrasound

Introduction

For the placental villosity formation it is necessary the formation of the placental vessels, that implies vasculo and angiogenesis phenomena. The vasculogenesis of the mesenchimal and imature villosity implies de novo formation of the blood vessels from the forerunner cells that were mesodermal derived. In the placentation time, the angiogenesis begins in the preexistent vascular bed; that represents the neovascularisation process based on the preexistent blood vessels, as an answer to the hypoxia or the tissues requests.

The vasculogenesis represents the process through which the primordial endothelial cells, the angioblasts, form the primitive vascular substratum. This process appears only during the fetal development, at the adult person the recruiting of the angioblasts on the backbone and peripheric blood appearing only as an ischemic damage effect.

It is necessary to establish some histological criteria for the normal vasculogenesis. The first capillary vessels appears at 18-20 days from the fecundation.(3)

We have proposed in this study also the imagistic, echographical examination and correlation of the trofoblast, by measuring its thickness at the embryo implantation site. Many authors (1) have studied the pregnancy prognostic by investigating the trofoblast in the first trimester and they have estimated nomograms of its thickness in the first trimester of pregnancy at the embryo implantation site.

Material and method

We have investigated 47 pregnant women in the first trimester which have been taken in the study when they presented for the pregnancy confirmation and consecutively asked for legal
abortion, with the gestational age between 5 and 12 weeks.

The diagnosis of pregnancy was established on the clinical and echographical data. There were expelled from the study the cases in which the curettage was made for medical reasons and the cases which had presented a developing pathology. There also expelled the cases that presented any affection that might have negative influence for the pregnancy development, the pregnant women with previous spontaneous abortion and those which related menstrual irregularities in the periconceptual period. For the same reasons, haven’t been not included in the study the pregnant women which presented at the ultrasonographic evaluation markers of an affected conception product, like anomalies of the gestational sac, anomalies of the yolk sac, retrochorionic haematoma, a discordant growth of the embryo correlated with the menstrual age, the bradicardy of the embryonic heart.

The patients were aged between 18 and 39 years, with a medium of 29 years.

The cases were grouped according to the clinical and echographical dates, calculating the gestational age in complete weeks (+/- 2-3 days). The histological study was made by collecting human placental tissue from the first trimester of pregnancy, the cases from the study being divided in 4 groups: 5-6 weeks of gestation, 7-8 weeks of gestation, 8-10 weeks of gestation, 10-12 weeks of gestation.

The histological method principle for the prelucration of the drown materials was based on the classical histological methods.

**Table nr. 1. Microanathomical and histochemical methods used in drown materials study**

<table>
<thead>
<tr>
<th>Nr. crt.</th>
<th>Method</th>
<th>Used abbreviation</th>
<th>Point out structure</th>
<th>Examination type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Hematoxylin-eosin</td>
<td>HE</td>
<td>General architecture of the tissues</td>
<td>Fotonic microscopy</td>
</tr>
</tbody>
</table>

**Basical principles of the methods used in the study**

<table>
<thead>
<tr>
<th>Nr. crt.</th>
<th>Method</th>
<th>Researched structure</th>
<th>Method principles</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>HE</td>
<td>General architecture</td>
<td>The use of a „basic“ colouring (Hematoxylin) and an „acid“ colouring (eosin)</td>
</tr>
</tbody>
</table>

For the ultrasonographic study we used the next eqipment: Sonoace 8000, Medison Co, Voluson 730 Pro and Logic P5, General Electrics, Alpha prosound, Aloka, and the evaluations in B mode and Doppler Color were made with the transvaginal sondes of this eqipments. For the ultrasonographic imagistical investigation of the trofoblast before the legal abortion at the pregnancies with gestational age between 6 and 12 weeks, had been evaluated the next parametres: the sonic age of the embryo – calculated according to CRL-crown lump length – and of the gestational sac, and the trofoblastic thickness-TT at the implantation site.

The implantation site location was made according to the gestational age: at 5-6 weeks of gestation by locating the yolk sac and eventually the omphalo-enteric duct; ulterior by determining the implantation site of the umbilical cord located by B mode (2D); in the cases when the location of the umbilical cord needed the color Doppler investigation (approximate 7 weeks of gestation), the value was preceded by the pregnant informed assent. It was defined and studied the correlation parameter of the trofoblastic thickness, established as the difference between the gestational age (measured in weeks) and the trofoblastic thickness at the implantation site (measured in mm). The seriated evaluations were made in order to estimate the predictive value about the gestational age; previous researches suggested absolute values under 3 associated with favourable evolution of pregnancy.

An informed assent was obtained from each patient and the proceedings had been approved by the The University of Medicine and Pharmacology Craiova Ethics Board.

The purpose of this study was the histological and imagistic – echographic investigation of the chorial villosity vascularisation developement and the trofoblast in the first trimester, trying to establish a histologic and imagistic grading, precisely important in the normal and pathologic pregnancy (recurrent abortion), and also to put up-to-date the placental villocities classification.

**Results and discutions**

There were noticed two processes in the vascular developement: the vasculogenesis and the angiogenesis.

The vasculogenesis involves the blood vessels development by the in-situ differentiation of the endothelial cells and the angiogenesis involves the development of the preexistent vessels.

The placental vasculogenesis begins immediately after the extraembryonic mesoderm invasion (10), the first structure of the tertiary villi appearing around the day 15 after conception. The vasculogenesis crosses 3 stages (2): the differentiation of the mesenchimal cells to angioblasts, the primary vessels asamblation in a primitive vascular network and the transition from
vasculogenesis to angiogenesis. During the vasculogenesis, it may be differentiated the first hemangioblastic cells lines, followed by the vascular lumen formation around day 28 after conception (15). Following the classical stages of the placental vasculogenesis, the first forerunners elements of the embryonic endothelium from the villositary lines, so called the hemangioblastic cells lines, had been demonstrated from the days 15-21 after conception.

With the day 28 after the conception begins the lumen formation surrounded by endothelial cells. In this phase, the hematopoietics stem cells become visible. After the primitive vascular network formation, the vasculogenesis performs to angiogenesis (4). After a primitive vascular bed is formed as a consequence of the vasculogenesis process(2), new vessels begin to form through the angiogenesis process, more exactly through burgeoning from the preexisting vessels.

The first blood islands appears in the mesoderm around the yolk sac wall in the third week of development, being made of mesodermic cells that differentiate and form hemangioblasts. The hemangioblasts formation is induced by VEGF (vascular endothelial growth factor), which is produced by mesodermic surrounding cells. The hemangioblasts from the center of the blood islands cells form the hematopoietic stem cells (the forerunners for all the blood cells), while the hemangioblasts from the periphery are differentiated in angioblasts (the forerunners for the blood vessels). This angioblasts proliferates under VEGF influence and becomes endothelial cells. The same factor regulate then the fusion of this endothelial cells that will form the primitive blood vessels (16).

In the first stages of the vasculogenesis, the grouped or singulare hemangioblastic cells had been shown in the mesenchimal villosity in the immature intermediary villosity, and apoptosis signs had been detected during the vascular lumen formation. The apoptosis phenomena had been remarqued arround the vascular pattern, with hematopoietical cells in various maturation stages.

The case analysis was made after the recrutation and grouping cases according to the gestational age, clinically and echographically established.

Studying the histological sections we studied the vasculogenesis stages cronology, that have shown the following manifestations: development and recruiting of the hemangioblastic forerunner cells, the formation of the hemangioblastic lines, the differentiation of the primitive endothelium, the primitive lumen formation, the endothelial tube elongation, the presence of the apoptotic bodies that shows the apoptosis, necessary for the vascular lumen formation.

![Graphic nr. 1. The cases distribution according to the gestational age](image)

In the cases with the gestational age between 5-6 weeks, we noticed a complexe network of hemangioblastic cells lines interconnected and connected to the citotrofoblast. The villosities appears dominated by a vascular elements network. The vessels and the lines are central and peripheric placed, in contact with the citotrofoblast. In this phase, the mesenchimal villosities are dominat, the actual name for the primary villus.(fig. 1A,B).

![Fig. nr.1 Mesenchimal villosities:hemangioblasts lines interconnected and connected to the trofoblast; ensemble(A) and detail(B) image Col HE](image)

In the cases with the gestational age between 7-8 weeks, the bitrofoblastic villosities presented a capillary network in way to differentiation and less hemangioblast cells lines. We noticed in the same time the presence of the mesenchimal
villous and the intermediary immature villous, the actual naming for the primary and secondary villi. (fig. nr. 2A, B)

![Fig. nr.2 Intermediare immature villous: capillary network in way to differentiation, rare hemangioblasts islands; ensemble(A) and detail(B) image Col HE](image)

In the cases with the gestational age between 9-10 weeks we noticed the presence of a differentiated vascular network central placed in general and the capillary network placed in the peripherical villous. The intermediary immature villous are dominant and the stem villous is differentiating now in monotrofoblastic villous; at its level will develop the angiogenesis phenomena. At this gestational age we can notice villous projections that contains capillary vessels and ends in glove finger according to the Lisman study (11). (fig. nr. 3A, B).

In the cases with the gestational age between 11-12 weeks, the stem villous are dominants, monotrofoblastic villous hanged with sincitiotrofoblast that presents a differentiated vascular network. The stem villous will be differentiated in mature intermediate villous that will develop to the terminal villous; this one characterises the villous tree of the second trimester placenta. (fig. nr. 4 A, B).

![Fig. nr.3 Stem villous: big vessels central placed with a lot of differentiated capillary vessels, apoptosis phenomena in the citotrofoblast; ensemble(A) and detail(B) image Col HE](image)

![Fig. nr. 4 Mature intermediate villous: monotrofoblastic villous with a differentiated vascular network; anucleated red blood cells; ensemble(A) and detail(B) image Col HE](image)

Through the echographical evaluation before evacuating the pregnancies we have noticed a normal correlation between the biometrical parameters of the first trimester pregnancy, respective between the yolk sac dimension and the crown rump length of the embryo, with a gestational sac diameter bigger with 5-7 mm in media than the CRL. This imagistic aspects
suggested normal evolutions for the studied pregnancies.

The study of the trofoblastic thickness at the implantation site showed the following values taking in consideration the gestational age. The data are illustrated in the graphic below:

The correlation parameter of the trofoblastic thickness showed values between -1.6 and +2.3, as we can see in the next table and graphic, proving itself like an acceptable evaluator of the gestational age. The results we obtained confirm the literature, which estimate for the normal evolutive pregnancies absolute values of this parameter under 3 (8).

### Tabel nr. 2. The distribution of the trofoblastic thickness values according to the gestational age

<table>
<thead>
<tr>
<th></th>
<th>6 w+</th>
<th>7 w+</th>
<th>8 w+</th>
<th>9 w+</th>
<th>10 w+</th>
<th>11 w+</th>
<th>12 w+</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.2</td>
<td>7.8</td>
<td>9</td>
<td>8.8</td>
<td>9.3</td>
<td>11.4</td>
<td>11.7</td>
<td></td>
</tr>
<tr>
<td>5.9</td>
<td>6.8</td>
<td>8.1</td>
<td>9.6</td>
<td>10</td>
<td>11.6</td>
<td>11.3</td>
<td></td>
</tr>
<tr>
<td>3.8</td>
<td>8.3</td>
<td>7.7</td>
<td>9.1</td>
<td>10.6</td>
<td>12.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.7</td>
<td>5.9</td>
<td>6.2</td>
<td>9.4</td>
<td>10.8</td>
<td>10.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.1</td>
<td>6.2</td>
<td>9.9</td>
<td>10.6</td>
<td>11.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.6</td>
<td></td>
<td>10.3</td>
<td>9.9</td>
<td></td>
<td></td>
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<tr>
<td>5.5</td>
<td></td>
<td>8.6</td>
<td>11.5</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>6.2</td>
<td></td>
<td>10.8</td>
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<td>7.4</td>
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</tr>
</tbody>
</table>

### Tabel nr. 3. The distribution of the values of the correlation parameters of the trofoblastic thickness according to the gestational age

<table>
<thead>
<tr>
<th></th>
<th>6 w+</th>
<th>7 w+</th>
<th>8 w+</th>
<th>9 w+</th>
<th>10 w+</th>
<th>11 w+</th>
<th>12 w+</th>
</tr>
</thead>
<tbody>
<tr>
<td>-1.2</td>
<td>-0.8</td>
<td>-1.1</td>
<td>-1.6</td>
<td>-1.3</td>
<td>-1.3</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>-0.5</td>
<td>-0.7</td>
<td>-0.4</td>
<td>-1.2</td>
<td>-0.7</td>
<td>-0.6</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>-0.2</td>
<td>0.5</td>
<td>0.1</td>
<td>0.8</td>
<td>0.5</td>
<td>0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.2</td>
<td>1.3</td>
<td>0.8</td>
<td>0.8</td>
<td>0.5</td>
<td>0.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.3</td>
<td>1.5</td>
<td>2.1</td>
<td>-0.1</td>
<td>0.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.6</td>
<td>0.2</td>
<td>0.7</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.7</td>
<td>0.5</td>
<td>0.9</td>
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<tr>
<td>0.9</td>
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<td>0.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

### Graphic nr. 2. The distribution of the trofoblastic thickness values according to the gestational age

### Graphic nr. 3. The distribution of the values of the correlation parameters of the trofoblastic thickness according to the gestational age
After obtaining the ultrasonographic and histological estimations, we made the correlation between the two types of parameters, histological and imagistical – echographic biometry. The concordance between the histological and imagistical aspects had been obvious in 37 cases, that means 92.5% from the studied cases with both methods, as we may see in the next tabel and graphic. We noticed a high concordance at any gestational age: 77.77% at 6 weeks+, 87.5 at 9 weeks+ and 100% at the rest of pregnancies.

**Tabel nr. 4. The concordance between histological and echographical biometry parameters**

<table>
<thead>
<tr>
<th>Number of cases</th>
<th>6w+</th>
<th>7w+</th>
<th>8w+</th>
<th>9w+</th>
<th>10w+</th>
<th>11w+</th>
<th>12w+</th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cases with histo-echographic concordance</td>
<td>7</td>
<td>5</td>
<td>5</td>
<td>7</td>
<td>7</td>
<td>4</td>
<td>2</td>
<td>37</td>
</tr>
<tr>
<td>The histo-echographic concordance percent</td>
<td>77.77</td>
<td>100</td>
<td>100</td>
<td>87.5</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>92.5</td>
</tr>
</tbody>
</table>

**Graphic nr. 4. The concordance between histological and echographical biometry parameters**

In two from the three cases of discordance between the histological evaluations and the imagistic ultrasonographical ones, the values of the correlation parameter of the trofoblastic thickness had been over 3 in absolute value.

Values under 3 in absolute value of the correlation parameter of the trofoblastic thickness had been after that correlated with the gestational age estimated according to the gestational sac and to the embryo biometry and historically confirmed, showing the next distribution:

**Tabel nr. 5. Correlation between: gestational age estimated according to the gestational sac biometry, the histological confirmation of the gestational age and the trofoblastic thickness index**

<table>
<thead>
<tr>
<th>The correlation parameter of the trofoblastic thickness</th>
<th>Histoechographical concordance (GS,CRL)</th>
<th>Total cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;3</td>
<td>yes</td>
<td>37</td>
</tr>
<tr>
<td>&gt;3</td>
<td>no</td>
<td>3</td>
</tr>
<tr>
<td>Total cases</td>
<td></td>
<td>40</td>
</tr>
</tbody>
</table>

According to this results, the statistic indicators recommend values under 3 in absolute value of the correlation parameter of the trofoblastic thickness as a true method to estimate the maximal gestational age, with a sensibility and a predictive positive value of 97.729%, a specificity and a negative predictive value of 66.666%, a false positive results rate of 27.027% and a false negative results rate of 33.333%, in normal low-risk pregnancy.

**Conclusions**

The vasculogenesis process determines a large network at which level all the vessels and the cells lines are connected at eachother. After this study, we realized a gestational grading correlated with the vasculogenesis process during the first trimester of pregnancy.

The morphological aspects of the mesenchimal villosity characterize the pregnancies with the gestational age between 5-6 weeks. Together with the pregnancy evolution, at 7-8 weeks gestational age, the vasculogenesis is correlated with the presence of the mesenchimal villositites and the appearance of the intermediare imature villosities. At 9-10 weeks gestational age, the imature intermediar villosities are dominant, with the persistence of the fetal citotrofoblasts and angioblasts. The pregnancies with the gestational age between 11-12 weeks are characterized by the presence (along the previous mentioned villosities) of the monotrofoblastic stem villositites, with finished vasculogenesis, capable of vasculary remodelation made by the angiogenesis.

This conclusions are necessary and can be used for establishing the pregnancy diagnosis at the patients with bleeding after a complete/incomplete abortion, and also for the „histological gestational age”, especially important in the absence of correlation between the clinical gestational age and the echographical gestational age.

Additional studies are necessary because there is few information about the exactly mechanism of appearance of the vasculogenesis and angiogenesis, which are the angiogenesis forerunner and that are the signals that induce these cells’ differentiation.

The correlation parameter of the trofoblastic thickness is an acceptable evaluator of the gestational age in the pregnancies with normal evolution, and the dates obtained by its investigation confirms the speciality literature, that estimates for the pregnancies with normal evolution absolute values of this parameter under 3.
The correlation of the histo-morphological villositary aspects of the normal evolutive pregnancies in the first trimester had been concordant in 92.5% cases with the dates of echographical biometry (GS, CRL), that allows us to affirm that sustained echographical examination of the pregnancy from the first trimester may give us clues regarding its evolution.

The statistic indicatories recommend a reserve under 3 in absolute value of the correlation parameter of the trofoblastic thickness as a good method to estimate the maximal gestational age, with a sensibility and a positive predictive value of 97.72%. These dates suggest in a way the possibility of including this parameter in the datation echography for the first trimester pregnancy and on the other way, the possibility that values outside the normality interval of this index may be associated with unfavourable evolutions of the pregnancies; for the confirmation of this theory are necessary further and larger studies.

References
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20.