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Ang2 immunoexpression vs vascular profile in chorio-villous germinative status in early-term compromised pregnancies

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Abstract

Background: The angiopoietin/TIE system is one of the signalling pathways that regulate vascular development and remodelling during morphogenesis of the placental complex. Deregulation of the vascularization process is associated with primary placental insufficiency through destabilization of the maternal-fetal functional system. Disruption of expression of pro-angiogenic factors is associated with reduction of the microcirculatory bed and installation of circulatory dysfunction.
The aim: Evaluation of Ang2 immunoexpression in early term compromised uterine pregnancies in the context of chorio-villous circulatory dysfunction in primary placental insufficiency.
Material and methods: Abortion product from 67 patients with early term compromised pregnancies (stagnant pregnancies – 43 cases, early miscarriages – 11 cases, control batch that included 13 cases of pregnancies solved on social indications) were immunohistochemically evaluated with the marker for anti-Ang2 and anti-CD31.
Results: The majority of sites analyzed were Ang2 negative, with the exception of syncytiotrophoblast, that reached the highest score (+3) in most cases. A maximum placental vascularisation index was achieved in the control group. The stagnant pregnacies batch has registered the lowest vascular density mean.
Conclusions: The placentation period is characterized by a weak Ang2 cellular environment in the chorio-villous germinal site in the group of short-term compromised pregnancies, except a highly positive syncytiotrophoblast. The immunoexpression profile in vascular endothelium correlates statistically significantly with placental vascularization index and chorio-villous vascular density in stagnant pregnancies.
Key words: Ang2, angiogenesis, fetal conceptus, compromised pregnancies, primary placental insufficiency.

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Introduction

Placental insufficiency is a syndrome caused by morpho-functional changes that lead to the incompetence of the placenta to maintain adequate metabolism between mother and fetus. The primary form of placental insufficiency is one of the types described and it occurs in the first trimester [1]. The first trimester of gestation is characterized by important successive phenomena on which the subsequent course of pregnancy will depend, namely: the first phase of cytotrophoblast invasion, implantation, placentation [2]. This period is of particular importance because any dysregulation of the above-mentioned phenomena under the influence of different factors (infectious, endocrine, genetic, etc.) contributes to the establishment of primary placental insufficiency, which is the main cause of spontaneous abortion and stagnant pregnancies [3].

The vascularization of chorionic villi is an important morphological indicator of the functional state of the placenta. It reflects the expression of metabolic processes between mother and fetus [4], and the physiological formation of the placental vascular network occurs under the action of various angiogenic factors.

The formation of such a competent vascular network in the early period is achieved by two successive processes: vasculogenesis (formation of vessels from endothelial progenitor cells) and angiogenesis (formation of vessels from pre-existing vessels) [5], the destabilization of which contributes to the onset of placental dyscirculatory failure.

The angiopoietin/TIE system is one of the signaling pathways that regulate vascular development and remodeling [6]. According to the molecular profile, 4 ligands (Ang1, Ang2, Ang3, Ang4) are elucidated. The first 2, as well as their tyrosine kinase-like receptors (TIE-1 and TIE-2), have a significant role in vascular morphogenesis of the placentation period [7]. The Ang1/ TIE-2 pathway promotes endothelial cell survival, endothelial integrity, anti-inflammatory and anti-apoptotic responses, which togeth-

er support reduced vascular permeability [8, 9]. Ang2 is an antagonist that competes with Ang1 in binding to TIE-2. The Ang2/ TIE-2 pathway reduces vessel stability and enhances vascular remodeling [10]. In experimental conditions, the effect of Ang2 in promoting endothelial cell survival, sprouting and migration has been demonstrated in a temporal and concentration-dependent manner [11-13]. The aforementioned ascribe to Ang2 a TIE-2-dependent agonist or antagonist effect [10, 14]. Angiopoietin 2 represents an endogenous ligand of the vascular endothelium-specific receptor tyrosine kinase Tie-2, which is expressed as early as the early period of placentogenesis with endo- and non-endothelial effects [15].

Vascular disturbances that could appear in the placentation period are frequently the cause of intrauterine developmental restriction [16, 17]. When assessing Ang2 levels in the first trimester of gestation, an association with risk of early-term miscarriage has been found [18]. Decreased expression of angiopoietin-2 in developmental restriction contributes to angiogenesis disturbances, mainly in the intermediate and terminal villi [19].

Deregulation of secretion and expression of pro-angiogenic factors during placentation is associated with disruption of vascular morphogenesis in the chorio-villous compartment by reduction of the chorio-villous fetal vascular bed, which makes this study of a great topicality.

Thus, the aim of the current study was to evaluate Ang2 immunoexpression in early-term compromised uterine pregnancies in the context of chorio-villous circulatory dysfunction in primary placental insufficiency.

Material and methods

Tissue samples obtained by uterine aspirate from 62 patients with early compromised pregnancies (3-12 weeks) were used. The specimens were collected at the Level III Perinatal Center, Institute of Mother and Child, during 2020. All patients were examined by ultrasonographic investigation and the gestational term was determined based on the first day of the last menstruation. The cases were grouped as follows: stagnant pregnancies (SP) – 43 cases, early miscarriages (EM) – 11 cases and pregnancies solved on social indication (SI) – 13 cases, last one being the control group. The age of the patients ranged from 22 to 40 years (mean±std. dev. being 30.5±5.6 years).

Clinical data were obtained from the medical records of each patient. The current research is part of a larger study of early-term compromised pregnancies within the state program "Morphological approach by conventional, histo- and immunohistochemical methods of the peculiarities of the pathological profile of early placentogenesis in early-term compromised pregnancies", code 20.80009.8007.17 P1P2 0750.

Cases were selected according to the inclusion and exclusion criteria:

Inclusion criteria: terminated pregnancies with gestational term from 3 to 12 weeks (clinically confirmed by ultrasound and terminated in the Institute of Mother and Child); pregnancies with pathological evolution: stagnant, early miscarriage; pregnancies with abortion at social indications/ desire; quality and volume of the aspirate: chorionic villi and decidual plates of a sufficient volume in standard paraffin blocks (1.0x1.0x0.5cm); monofetal pregnancies; no age threshold.

Exclusion criteria: serious somatic pathology; multiple pregnancies; pregnancies terminated on medical indication; lack of clinical-anamnestic data in medical records; lack of gestational term specification and ultrasound confirmation of pregnancy status.

The examination included histoprocessing of tissue samples, application of the usual histological method (haematoxylin-eosin), immunohistochemical method (anti-CD31; anti-Ang2) with evaluation of histopathological features and immunoexpression, and statistical analysis.

Primary processing. Tissue material of the conception product was collected in a short time in obstetric department with rapid fixation in 10% formalin, pH 7.2-7.4, to reduce the risk of early lysis of tissue material and bacterial flora growth. The fixation period in 10% buffered formalin solution was 24 hours. The paraffin embedding system was DP500/CIT2002 (Bio-Optica, Italy). Histochemical and histological processing of samples was performed on the histoprocessor "TISSUE-TEK, VIP 6AI" (Sakura, Japan), sectioning on the HM325 microtome (Thermoscientific) (USA). 3.5 μm thick sections were placed on positively charged slides (APTACA, Italy).

Histological method. Sections were stained by the conventional classical hematoxylin-eosin (H.E.) method using Mayer hematoxylin (HEMM-36/21, BIOGNOST, Slovenia) and 1% Y eosin (EOY10-35/21, BIOGNOST, Slovenia). Sections for H.E. were automatically stained with the AUS-240 autostainer, (Bio-Optica, Italy) and automatically mounted (TISSUE-TEK, Clas™, Sakura, Japan). Suitable sections (sufficient tissue material) were selected for immunohistochemical staining.

Immunohistochemical method. Immunohistochemical assays included manually adopted operational procedures for anti-Ang2 (ab5630) antibodies with the application of the Novolink™MaxPolimer detection system, Leika (RE7280-K) [20] and anti-CD31 (JC70A) with the application of the EnVision™FLEX detection system, high pH (K8000) [21]. The conventional immunohistochemical method was applied (Table 1). Deparaffinization was performed in two toluene baths (code UN1294, Sigma-Oldrich), the first bath for 60 min at 59°C in thermostat, followed by the second bath for 5 min at room temperature. Slides were then placed in a mixed bath of toluene and 96% alcohol for 5 min, then – 2 baths of 96% alcohol with 2 rehydrations of 10 min each in distilled water. For the purpose of epitope unmasking, sections intended for application of Ang2 and CD31 antibody were exposed to dissolved Target Flex solution (1ml Target: 49 ml distilled water) at high pH, 20 minutes exposure time at 95°C–96°C with a total pretreatment and posttreatment time of 60 minutes.

Table 1. Antibodies used: source, dilution, unmasking system, detection system, incubation time

Antibody / clone	Sourse/ incubation time/ dilution	Retrieval system / time	Detection system / time
CD31 JC70A	Abcam, Cambridge, UK/ 20 min/ ready- to-use	Solution Target Flex, high pH/ Water bath at 95°C - 96°C / 20 min	EnVision™FLEX, high pH
Ang2 ab56301	Abcam, Cambridge, UK/ 12 hours/ 1:1000	Solution Target Flex, high pH/ Water bath at 95°C - 96°C / 20 min	Novolink™MaxPolimer, Leika / 30 min

Neutralization of endogenous peroxidase was performed with peroxidase block for 7 minutes followed by incubation with Novocastra Protein Block for 5 minutes. Next step was incubation with primary antibody (anti-Ang2) for 12 hours at +4°C, 1:1000 dilution, including 5 minutes in thermostat at 59°C. In the case of anti-CD31 antibody, incubation lasted for 20 minutes at room temperature. After incubation with the primary antibody, neutralization of endogenous peroxidase with peroxidase block was performed for 5 minutes, followed by application of the secondary antibody (HRP) for 20 minutes and DAB (3,3'-diaminobenzidine) applied as a chromogenic substrate for 5 minutes. Counterstaining of nuclei was performed with Leica haematoxylin (RE7164) when using the Novolink™MaxPolimer detection system and with Mayer's haematoxylin (HEMM-36/21, BIOGNOST, Slovenia) when using the EnVision™FLEX system. The final product of the reaction was stained brown with cytoplasmic patern for Ang1 and membranous patern for CD31. Then, the histological slide panel was subjected to the dehydration and clarification procedure using two absolute alcohol baths, one alcohol and toluene mixed bath and three toluene exposures, each exposure being 5 minutes. The final procedure consisted of mounting the slides with BMC-100 solution. In the manual immunohistochemical staining procedure, Sequenza™ Immunostaining Center was applied using Thermo Shandon Coverplat.

Microscopic evaluation. The CD31 protein (endothelial cell adhesion receptor) was detected at the membrane level, manifested by the presence of brownish colour in the tissue studied. In all sections, blood vessels were quantified by the hot-spot method, the technique having been described in detail in a previous study. For the assessment of Ang2 immunoexpression, initially, areas with the highest density of chorionic villi were determined at ×100 magnification. Immunoexpression was assessed at ×200 magnification using the semiquantitative hot-spot method applied to three representative areas of the germinal site corresponding to chorionic villi (vascularized and avascularized). For the evaluation of anti-Ang1 immunoexpression, the scoring system based on the intensity of immunoreactivity was applied. The reaction was considered to be positive in the presence of brown color in the tissue studied according to the specificity of each antibody. The following score was applied: 0 (no staining); +1 (weak but detectable staining); +2 (moderate or distinct staining); +3 (strong or pronounced staining). The reaction for anti-Ang2 was analyzed in the following areas: trophoblast, mesenchymal stromal cells, angiogenic/ endothelial vascular cells.

The above-mentioned structures were counted in each of the 3 study groups (SP, EM, SI), grouped according to gestational term into the following groups: 3-5 weeks, 6-9 weeks and 10-12 weeks. Quantification of positive cells was performed on the Axio Imager A2 microscope (Carl Zeiss, Germany) equipped with the AXIOCam MRc5 recording camera.

Data analysis. Statistical procedures (Winstat 2012.1, R. Fitch Software, Bad Krozingen, Germany) included determination of the Spearman's rank correlation coefficient (Spearman (rs)) and differences between groups and subgroups (Mann-Whitney U test). Results were considered statistically significant at $p<0.05$.

Results

The study was carried out on a group of 67 cases of early compromised pregnancies, divided into: stagnant pregnancies – 43 cases (64.17%), early miscarriages – 11 cases (16.41%) and the control batch that included 13 cases of pregnancies solved on social indications/ desire (SI) (19.4%).

The intensity of Ang2 immunoexpression varied from 0 to +3 and can be analyzed in the tables 2, 3 and 4.

The majority of sites analyzed in the control group were

Table 2. Immunoexpression intensity of anti-Ang 2 (control batch)

GT	CV-cyto-tropho				CV-syn-tropho				CV-vasc-endot				CV-hofbauer				CV-stroma			
	0	**+1**	**+2**	**+3**	**0**	**+1**	**+2**	+3	0	+1	+2	+3	0	+1	+2	+3	0	+1	+2	+3
3-5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6-9	6	0	0	0	0	0	0	**6**	**6**	0	0	0	**6**	0	0	0	**6**	0	0	**0**
10-12	7	0	0	0	0	0	0	**7**	**7**	0	0	0	**7**	0	0		**7**	0	0	**0**
Total	**13**	0	0	0	0	0	0	**13**	**13**	0	0	0	**13**	0	0	0	**13**	0	0	0

Note: GT – gestational term, CV-cyto-tropho – chorionic villi cytotrophoblast, CV-syn-tropho – chorionic villi syncytiotrophoblast, CV-vasc-endot – chorionic villi vascular endothelium, CV-hofbauer – chorionic villi Hofbauer cells, CV-stroma – chorionic villi stroma. Immunoexpression intensity was rated as follows: 0 (absent); +1 (weak); +2 (moderate); +3 (pronounced). The highest values were marked as **bold**.

Table 3. Immunoexpression intensity of anti-Ang 2 (SP batch)

GT	CV-cyto-tropho				CV-syn-tropho				CV-vasc-endot				CV-hofbauer				CV-stroma			
	0	**+1**	**+2**	**+3**	**0**	**+1**	**+2**	**+3**	**0**	**+1**	**+2**	**+3**	**0**	**+1**	**+2**	**+3**	**0**	**+1**	**+2**	**+3**
3-5	**8**	0	0	0	0	0	2	**6**	**4**	0	1	3	**8**	0	0	0	**8**	0	0	0
6-9	**26**	0	0	0	0	0	1	**25**	**10**	8	4	3	**20**	0	2	0	**25**	0	1	0
10-12	**9**	0	0	0	1	0	0	**8**	**4**	4	0	0	**3**	3	2	0	**5**	3	0	1
Total	**43**	0	0	0	1	0	3	**39**	**18**	12	5	6	**31**	3	2	0	**38**	3	1	1

Note: SP – stagnant pregnancies, GT – gestational term, CV-cyto-tropho – chorionic villi cytotrophoblast, CV-syn-tropho – chorionic villi syncytiotrophoblast, CV-vasc-endot – chorionic villi vascular endothelium, CV-hofbauer – chorionic villi Hofbauer cells, CV-stroma – chorionic villi stroma. Immunoexpression intensity was rated as follows: 0 (absent); +1 (weak); +2 (moderate); +3 (pronounced). The highest values were marked as **bold**.

Table 4. Immunoexpression intensity of anti-Ang 2 (EM batch)

GT	CV-cyto-tropho				CV-syn-tropho				CV-vasc-endot				CV-hofbauer				CV-stroma			
	0	**+1**	**+2**	**+3**	**0**	**+1**	**+2**	**+3**	**0**	**+1**	**+2**	**+3**	**0**	**+1**	**+2**	**+3**	**0**	**+1**	**+2**	**+3**
3-5	**2**	0	0	0	0	0	1	**1**	**1**	1	0	0	**1**	1	0	0	**2**	0	0	0
6-9	**7**	0	0	0	0	0	0	**7**	**6**	0	1	0	**5**	0	0	0	**7**	0	0	0
10-12	**2**	0	0	0	0	0	0	**2**	0	**2**	0	0	**1**	1	0	0	**1**	0	1	0
Total	**11**	0	0	0	0	0	1	**10**	**7**	3	1	0	**7**	2	0	0	**10**	0	1	0

Note: EM – early miscarriages, GT – gestational term, CV-cyto-tropho – chorionic villi cytotrophoblast, CV-syn-tropho – chorionic villi syncytiotrophoblast, CV-vasc-endot – chorionic villi vascular endothelium, CV-hofbauer – chorionic villi Hofbauer cells, CV-stroma – chorionic villi stroma. Immunoexpression intensity was rated as follows: 0 (absent); +1 (weak); +2 (moderate); +3 (pronounced). The highest values were marked as **bold**.

Ang2 negative, with the exception of syncytiotrophoblast, that reached the highest score (+3) in 100% cases. Cytotrophoblast was totally Ang2 negative in SP and EM groups. The Hofbauer cells and the stromal ones showed different scores (SP: 70.4/ EM: 63.6%; SP: 86.3/ EM: 90.9%, respectively) (tab. 3, 4). Endothelial cells were Ang2 positive in 56.1% of SP cases and in 63.6% of EM cases. The syncytiotrophoblast was Ang2 positive in most of cases of all groups studied: 90.6% in SP batch and 90.9% in EM batch (fig. 1).

Then was analyzed the different Ang2 immunoreactivity between batches and inside batches by applying the Mann-Whitney U test. Thus, were obtained the following statistically significant differences of endothelial cells immunoreactivity between groups: SP 6-9 weeks vs ASI 6-9 weeks (p=0.01); SP 10-12 weeks vs ASI 10-12 weeks (p=0.04); ASI overall cases vs SP overall cases (p=0.001) and overall EM cases vs ASI overall cases (p=0.025). At the same time, differences were attested in the Hofbauer cell compartment: SP 10-12 weeks and ASI 10-12 weeks (p=0.02); SP 10-12 weeks and SP 6-9 weeks (p=0.005). Were also identified variations in the stromal areas: SP 10-12 weeks vs SP 6-9 weeks (p=0.003). Statistically significant differences in the ASI batch were not attested.

Subsequently, to assess the involvement of Ang2 expression in the morphogenesis of the chorio-villous vascular network, placental vascularization index (PVI, %) and chorio-villous vascular density (VD, %) were determined with the application of anti-CD31 antibody. According to the results obtained, anti-CD31 immunoexpression in the

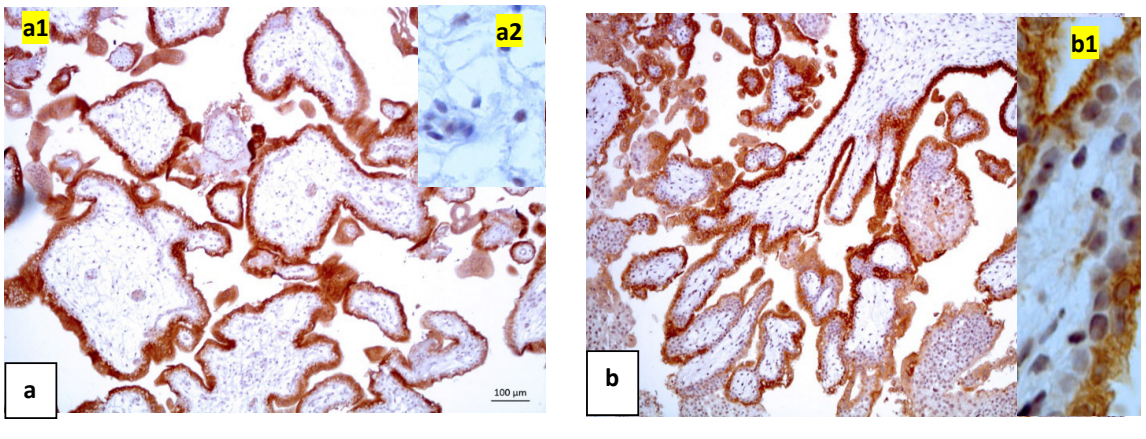

Fig. 1. Differential Ang2 immunoexpression in choriovillar cell profile in early compromised pregnancies: a) SI - a1 (syncytiotrophoblast - score +3, cytotrophoblast - 0) and a2 (stroma - 0); b) EM – b1 (cytotrophoblast - 0; syncytiotrophoblast - score +3; endothelium - score +1). Immunoreaction for anti-Ang2, DAB; a1, b1 ×100; a2, b2 ×400.

Table 5. Descriptive statistical analysis of placental vascular density

Vascular profile	ASI batch (M±SD), weeks			EM batch (M±SD), weeks			SP batch (M±SD), weeks		
	3-5	6-9	10-12	3-5	6-9	10-12	3-5	6-9	10-12
PVI%	-	92.08 ±7.67	90.52 ±12.08	97.18 ±3.98	73.39 ±12.98	78.58 ±23.7	64.12 ±33.58	51.72 ±27.24	55.44 ±32.24
PVI_{total} %	-	91.3±9.87		83.05±13.5			57.09±31.02		
VD%	-	7.15 ±1.38	11.91 ±9.47	7.23 ±0.43	7.26 ±3.1	16.91 ±2.24	4.87 ±1.77	5.53 ±2.99	6.52 ±5.8

Note: M – mean; SD – std. Dev.; GT – gestational term, weeks; ASI – abortion on social indications; EM – early miscarriage; SP – stagnant pregnancies; PVI – placental vascularization index; VD – chorio-villous vascular density.

chorionic villous stroma determined a maximum IVP in the control group (ASI) (91.3±9.87). The values were lower in EM and SP batches (83.05±13.5 and 57.09±31.02, respectively). The maximum VD mean values were determined in the EM and ASI groups (10.46±1.92 and 9.53±5.42, respectively). The SP batch has registered the lowest VD mean (5.64±3.52) (tab. 5, fig. 2).

Next step was to analyze the VD and PVI differencies between batches, grouped by gestational term. The correlations can be seen in table 6.

Table 6. Mann-Whitney U test results, intergroup analysis

GT, weeks	Batch	Correlations obtained for the VD	Correlations obtained for PVI
6-9 n=38	ASI vs EM	p= 0.668	**p=0.01**
	ASI vs SP	**p= 0.016**	**p=0.001**
	EM vs SP	p=0.116	p=0.059
10-12 n=20	ASI vs EM	p=0.076	p=0.236
	ASI vs SP	p=0.189	**p=0.006**
	EM vs SP	p=0.143	p=0.242
3-5 n=9	EM vs SP	p=0.142	p=0.106

Note: GT – gestational term, weeks;ASI – abortion on social indications; EM – early miscarriage; SP – stagnant pregnancies; PVI – placental vascularization index; VD – chorio-villous vascular density. Statistically significant correlations were marked as **bold**.

The CD31 immunoreactivity of endothelial cells was directly dependent on the PVI in the SP group (rs=0.67, p=0.04). Statistical analysis of data also showed some statistically significant correlations between VD and the CD31 expression by endothelial cells (SP batch, 3-5 weeks and 10-12 weeks) (rs=0.85, p=0.003 and rs=0.65, p=0.04, respectively). VD was also dependent on the immunoreactivity of Hofbauer cells and stromal cell of chorionic villi (SP batch) (rs=0.36, p=0.01 and rs=0.31, p=0.02, respectively). The SI and EM batches did not show any statistically significant correlations.

Discussion

The first trimester of gestation is an important one due to the formation of the vascular capillary network and the large vessels, arterioles and venules, which are of particular importance in the establishment of the blood supply to ensure the requirements of the growing embryo [22, 23]. Thus, the formation of blood vessels in the chorionic villi, which happens in the first trimester of gestation, is fundamental in the establishment of full metabolic processes between mother and embryo/fetus, which favors the physiological progress of pregnancy.

The vascularization of the chorionic villi is an important morphological indicator of the functional state of the placenta in achieving this connection [4], and the physiological formation of the placental vascular network oc-

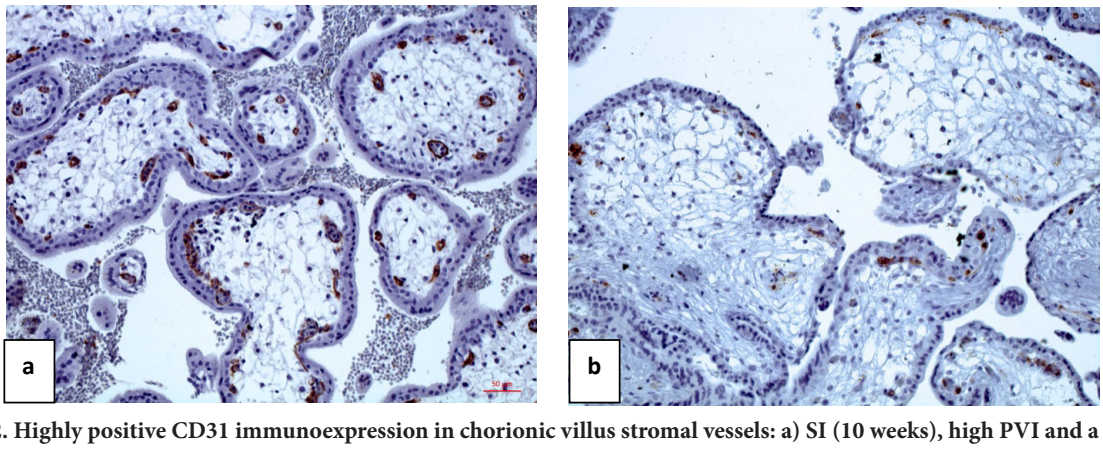

Fig. 2. Highly positive CD31 immunoexpression in chorionic villus stromal vessels: a) SI (10 weeks), high PVI and a reach blood network and b) SP (12 weeks), low PVI, hypovascularized villi. Immunoreaction for anti-CD31, DAB; a, b x100.

curs under the action of various angiogenic factors, among which an important role is attributed to the angiopoietins family.

Angiopoietins are a family of extracellular ligands, integral proteins involved in angiogenesis and vascular remodelling. The angiopoietin/TIE system is one of the signaling pathways that regulate vascular development and remodeling [6]. According to the molecular profile, 4 ligands (Ang1, Ang2, Ang3, Ang4) are elucidated, the first two, as well as their receptors (TIE-1 and TIE-2) having significance in the vascular morphogenesis of the placental period. [7].

Ang2 contains 496 aminoacids and 60% of it is homologous to Ang1 [24]. Both ligands have a similar structure with different biological functions and act through the common tyrosine kinase receptor (TIE-2) [10, 14]. The effect of Ang2 is contradictory and depends on the presence or absence of VEGF, which may convert its anti-angiogenic role into a proangiogenic one. Thus, Ang2 is considered to be an antagonist for Ang-1 when binding to the TIE-2 receptor that leads to a reduced vascular stability through destabilization with subsequent restructuring [10].

Therefore, Ang2 immunoexpression in chorio-villous germinal status was assessed by immunohistochemical investigation with determination of angiogenic profile. Were evaluated the localization and degree of immunoexpression (tab. 2, 3, 4). According to the results obtained, a differentiated immunoexpression was established both based on the cellular profile and in relation to the batch of early compromised pregnancies. Were found out some statistically significant differences, with a predominance of negative immunoexpression and a highly positive (score +3) syncytiotrophoblast.

In order to assess the proangiogenic effect in the early period of vascular capillary bed formation in the stroma of developing chorionic villi, cellular immunoexpression data were correlated with the placental vascularization index. Statistically significant positive correlation was attested only in the SP group, 3-5 weeks of gestation, in the endothelial cell compartment (rs=0.67, p=0.04). The batch of SI/D and the EM one showed no intragroup correlations. Thus, differential immunoexpression in the cellular compartment denotes early-term Ang2 involvement in reducing placental vascular index.

As a confirmation of this hypothesis, when analysing immunoexpression in the research sites vs chorio-villous vascular density, statistically significant correlations were found in the SP group, 3-5 and 10-12 weeks, between endothelial cell and stromal/mesenchymal cell components as well as Hofbauer cells for the SStotal group.

The Ang2 expression is different during pregnancy, i. e., in the early period it is high, whereas later it decreases. This was proved by assessing serum levels of these proteins in normal pregnancies, including mRNA determination [15]. According to one study, the authors have noticed the presence of Ang2 in the early period of placentalogenesis (4th week) in the trophoblast, particularly in the syncytiotrophoblast [25]. At the same time, various studies are presented reporting a variety of Ang2 expression and its involvement in various pathologies, such as growth restriction, pre-eclampsia etc. [19], which are not the aim of investigation in this paper. The results are consistent with the authors' data supporting differential cellular immunoexpression of Ang2 in the placental parenchyma, including diversity in the degree of expression in early pregnancies [25].

Conclusions

The placentation period is characterized by a weak Ang-2 cellular environment in the chorio-villous germinal site in the group of short-term compromised pregnancies, except a highly positive syncytiotrophoblast. The immunoexpression profile in vascular endothelium correlates statistically significantly with placental vascularization index and chorio-villous vascular density in stagnant pregnancies.

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EC drafted the first manuscript, conducted the laboratory work; VF and LS interpreted the data; LiSi collected the material; VD designed the study and revised the manuscript criticaly. All the authors reviewed and approved the final version of the manuscript.

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Ethical approval and consent to participate
No approval was required for this study.

Conflict of interests
No competing interests were disclosed.