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Ang1 immunoexpression vs vascular profile in chorio-villous germinative status in early term compromised pregnancies

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Abstract

Background: A normal maternal-fetal system is essential for the functioning of the placenta during placentation and the establishment of the maternal-embryo-fetal vascular circulation. The angiopoietin/TIE pathway is involved in vascular morphogenesis through regulation, survival and maturation of endothelial cells concomitant with vascular remodeling. Deregulation of pro-angiogenic factor secretion and expression is associated with disruption of vascular morphogenesis, reduction of vascular bed and installation of primary placental insufficiency. The aim: Evaluation of Ang 1 immunoexpression in early term compromised pregnancies in the context of chorio-villary circulatory dysfunction in primary placental insufficiency.
Material and methods: Abortion product from 61 patients (stagnant pregnancies – 39 cases, early miscarriage – 8 cases, control group – 14 cases of pregnancies solved on social indications/ desire) were immunohistochemically evaluated with the marker for anti-Ang1 and anti-CD31.
Results: The villous syncytiotrophoblast was the most immunoreactive area. Most of cases of the pregnancies terminated on social indications/ desire were anti-Ang1 negative. The levels of anti-Ang1 immunoexpression were statistically significantly different in case of syncytiotrophoblast of early miscarriages and abortions terminated on social indications/ desire. The highest chorio-villous vascular density was noticed in the abortions on social indications/ desire and early misscariages group.
Conclusions: The placental period is characterized by a weak angiogenic Ang1 differentiated cellular environment in the chorio-villous germinal site in the group of short term compromised pregnancies. The selectively immunoexpressed cellular profile statistically significantly correlates with placental vascular index and chorio-villous vascular density in stagnant pregnancies.
Key words: Ang1, angiogenesis, fetal conceptus, compromised pregnancies, primary placental dysfunction.

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Introduction

Establishing of a functional maternal-fetal system is essential for the functioning of the placenta during placentation with the establishment of maternal-fetal vascular circulation and reduction of pregnancy complications. The vascularization of chorionic villi is an important morphological indicator of the functional state of the placenta, reflecting the expression of metabolic processes between mother and fetus [1]. 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) [2], the destabilization of which contributes to the onset of placental circulatory failure.

The primary placental insufficiency is of particular impact in the development of the human conceptus in prenatal pathology. It is caused by morpho-functional changes and is characterized by lack of or decreased optimal mother-embryo/fetus exchange during the establishment of hemochorionic blood circulation. According to the study by Regnault T. R. et al. (2002), defective angiogenesis is often the cause of intrauterine developmental restriction [3], as a result of vascular disturbances [4].

Angiogenic factors of the angiopoietin family play an important role in stabilizing of the placento-fetal vascular network during placentation. According to the molecular profile, 4 ligands (Ang1, Ang2, Ang3, Ang4) are elucidated, the first two and their tyrosine kinase-like receptors (TIE-1 and TIE-2) having a significant role in vascular morphogenesis during placental period [5]. The regulatory effect of Ang1 on endothelial cell survival and maturation occurs via its specific receptor tyrosine kinase (TIE-2) [6]. Thus, angiopoietins 1 are endogenous ligands of the vascular endothelial-specific tyrosine kinase receptor TIE-

2, which is still expressed in the early period of placentation and has endothelial and non-endothelial effects [7].

At the same time, the expression of angiopoietins is diverse and contradictory, often being elucidated much later in the pathogenesis of pathologies, such as intrauterine developmental restriction, pre-eclampsia, etc. [7-9]. In this context, Schneuer F. J. et al. (2013) found an association with risk of miscarriage at gestational term greater than 10 weeks while evaluating the impact of angiopoietin1 levels in the first trimester of pregnancy [10].

Deregulation of secretion and expression of pro-angiogenic factors during placentation may be associated with disruption of vascular morphogenesis in the chorio-villous compartment because of endothelial dysfunction and reduction of the chorio-villous fetal vascular bed. The above-mentioned ideas make this study of a high topicality.

Thus, the aim of the current study was to assess Ang1 immunoexpression in early term compromised uterine pregnancies in the context of chorio-villous circulatory dysfunction.

Material and methods

Tissue samples were obtained by uterine aspirate from 61 patients with early compromised pregnancies (3-12 weeks). 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) – 39 cases, early miscarriages (EM) – 8 cases and pregnancies solved on social indication/desire (SI/D) – 14 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-Ang1) with evaluation of histopathological features and immunoexpression, and statistical processing.

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-Ang1 (ab8451) antibodies with the application of the Novolink™MaxPolimer detection system, Leika (RE7280-K) [11] and anti-CD31 (JC70A) with the application of the EnVision™FLEX detection system, high pH (K8000) [12]. 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 Ang1 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 post-treatment time of 60 minutes. 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-Ang1) 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

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

Antibody / clone	Source/ 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
Ang1 ab8451	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

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 pattern for Ang1 and membranous pattern 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. For the assessment of Ang1 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-Ang1 was analyzed in the following areas: cytosyncytiotrophoblast, mesenchymal stromal cells, angiogenic/ endothelial vascular cells.

The above-mentioned structures were counted in each of the 3 study groups (SP, EM, SI/D), 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 61 cases of early terminated pregnancies, divided into: stagnant pregnancies – 39 cases (82.9%), early miscarriage – 8 cases (17.1%) and the control batch that included 14 cases of pregnancies solved on social indications/ desire (SI/D) (24.2%).

The intensity of Ang1 immunoexpression varied from 0 to +3 and can be analyzed in the tables 2, 3 and 4.

Table 2. Immunoexpression intensity of anti-Ang 1 (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	0	6	1	0	0	6	0	0		4	3	0	1	1	2	0	1	4	2	0
6-9	5	15	4	0	1	18	**5**	0	2	18	4	0	5	15	3	0	5	**17**	2	0
10-12	6	2	0	0	0	**8**	0	0	5	2	1	0	4	3	1	0	6	1	1	0
Total	11	**23**	5	0	1	**32**	5	0	7	**24**	8	0	10	**19**	6	0	12	**22**	5	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-Ang1 (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	0	0	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0
6-9	2	**3**	0	0	0	0	**5**	0	2	**3**	0	0	2	3	0	0	2	**3**	0	0
10-12	**2**	0	0	0	0	**2**	0	0	1	1	0	0	1	1	0	0	2	0	0	0
Total	**4**	3	1	0	0	2	**6**	0	**3**	4	0	0	**3**	4	0	0	**4**	3	1	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 4. Immunoexpression intensity of anti-Ang 1 (SI/D 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	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6-9	**4**	2	1	0	0	**4**	3	0	**4**	1	2	0	**6**	1	0	0	4	1	2	0
10-12	**6**	2	0	0	3	**5**	0	0	**5**	3	0	0	**7**	1	0	0	4	4	0	0
Total	**10**	4	1	0	3	**9**	3	0	**9**	4	2	0	**13**	2	0	0	**8**	5	2	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**.

In most cases of stagnant pregnancies (SP) (tab. 2), the anti-Ang1 immunoexpression was given the +1 score. The villous syncytiotrophoblast was the most immunoreactive area, the +1 score being assigned in 94.8% of cases. In the early miscarriage (EM) batch (tab. 3), the syncytiotrophoblast was given the +1 score in 75% of cases, and the +2 score was much less frequent (fig. 1). Most of cases of the contol batch (SI/D) were anti-Ang1 negative. Again, the most immunoreative region was the syncytiotrophoblast. To be mentioned that the +3 score was never assigned in any study group.

Then were analyzed the differencies between groups by applying the Mann-Whitney U test. The levels of anti-Ang1 immunoexpression were statistically significantly different in case of syncytiotrophoblast of EM and SI/D (p=0.02). Intragroup statistically significant differencies were also noticed, particularly in case of SP 10-12 weeks vs SP 3-5 weeks: cytotrophoblast (p=0.004) and vascular endothelium (p=0.02); SP 10-12 weeks vs SP 6-9 weeks: cytotrophoblast, vascular endothelium and stroma (p=0.01, p=0.02 and p=0.03, respectively).

Subsequently, to assess the involvement of Ang1 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 chorionic villous stroma determined a maximum PVI mean in the control group (SI/D) (91.72±9.5). The means of PVI in EM and SP

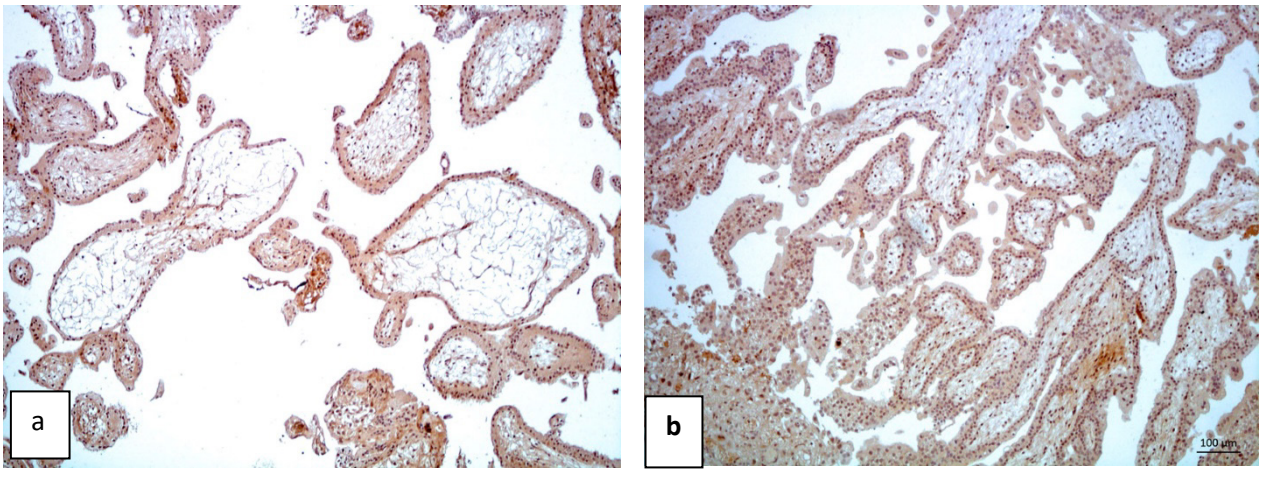

Fig. 1. Differential immunoexpression in the chorio-villous cell profile in early term (6 weeks) compromised pregnancies: a) SP and b) EM. Anti-Ang1 immunoreaction, DAB; x100

Table 4. Descriptive statistical analysis of placental vascular density

Vascular profile	SI/D batch (M±SD), GT			EM batch (M±SD), GT			SP batch (M±SD), GT		
	3-5	6-9	10-12	3-5	6-9	10-12	3-5	6-9	10-12
PVI%	-	92.48 ±7.76	90.97 ±11.25	100 ±0.00	68.1 ±11.39	78.58 ±23.71	70.69 ±29.21	52.64 ±26.83	56.50 ±36.44
PVI% total	-	91.72±9.5		82.22±17.55			59.94±30.82		
VD%	-	6.61 ±1.2	12.1 ±8.78	7.54 ±0.00	6.2 ±3.07	16.91 ±2.24	5.23 ±1.73	5.5 ±3.0	6.79 ±5.97
VD% total	-	9.36±4.99		10.21±2.65			5.84±3.56		

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

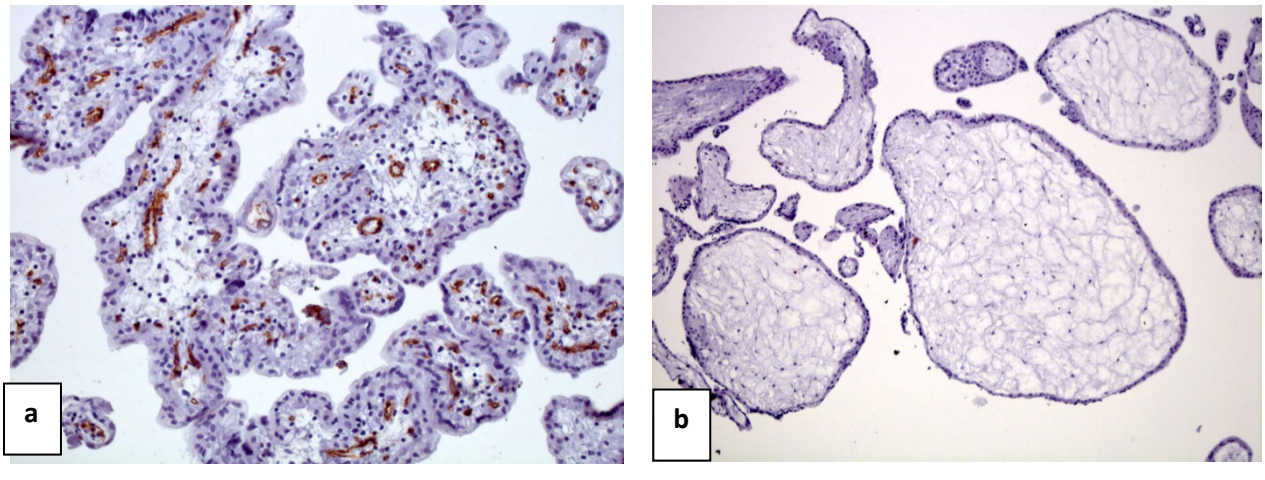

Fig. 2. High positive immunoexpression in stromal vessels of chorionic villi of early compromised pregnancies: a) SI/D (11 weeks) with high PVI and rich vascular profile vs b) SP (10 weeks) with low PVI with virtually avascular chorionic villi. Anti-CD31 immunoreaction, DAB; x100.

batches were 82.22±17.55 and 59.94±30.82, respectively. The highest chorio-villous vascular density was noticed in the SI/D and EM group (9.36±4.99 and 10.21±2.65, respectively). The lowest density was observed in case of SP (5.84±3.56) (tab. 4, 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 5.

The PVI was inversely dependent on chorionic villi stroma (rs=-0.40, p=0.03) in the 6-9 weeks group. However, same correlation was positive in the 10-12 weeks group (rs=0.76, p=0.01). The EM batch was characterized by a high overall PVI in the syncytiotrophoblast (rs=0.88, p=0.01) (tab. 6). There were no statistically significant correlations in the SI/D batch.

In the case of the statistical analysis of chorio-villous vascular density statistically significant positive correlations were established in the SP group with gestation term of 10-12 weeks, particularly in the vascular endothelium area, Hofbauer cell compartment, and stromal/mesenchymal site (rs=0.87, p=0.002; rs=0.73, p=0.02 and rs=0.65, p=0.04, respectively) (tab. 7). The group of uterine pregnancies solved on social indications/ desire and in early miscarriages showed no statistically significant intragroup correlations.

Table 5. Mann-Whitney U test results, intergroup analysis

GT, weeks	Batch	Correlations obtained for the VD	Correlations obtained for PVI
6-9	SI/D vs EM	p=0.855	**p=0.011**
	SI/D vs SP	**p= 0.021**	**p=0.001**
	EM vs SP	p=0.453	p=0.294
10-12	SI/D vs EM	p=0.086	p=0.283
	SI/D vs SP	p=0.183	**p=0.006**
	EM vs SP	p=0.117	p=0.296
3-5	EM vs SP	p=0.317	p=0.207

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

Table 6. Statistically significant correlations of PVI in different areas in the study groups

Batch	GT, weeks	Area studied	r_s	p
SP	6-9	CV-stroma/ mesenchyme	-0.40	0.03
	10-12	CV-stroma/ mesenchyme	0.76	0.01
EM	total	CV-syn-tropho	0.88	0.01

Note: GT – gestational term, CV-stroma – chorionic villi stroma, CV-syn-tropho – chorionic villi syncytiotrophoblast, SP – stagnant pregnancies, EM – early miscarriage, rs – Spearman's correlation coefficient. The results were considered statistically significant in case of $p<0.05$.

Table 7. Statistically significant correlations between choriovascular density and different areas in the study groups

Batch	GT, weeks	Area studied	r_s	p
SP	10-12	CV-vasc-endot	0.87	0.002
		CV-hofbauer	0.73	0.02
		CV-stroma/ mezenchim	0.65	0.04

Note: GT – gestational term, SP – stagnant pregnancies, CV-vasc-endot – chorionic villi vascular endothelium, CV-hofbauer – chorionic villi Hofbauer cells, CV-stroma – chorionic villi stroma, , rs – Spearman's correlation coeficient. The results were considered statistically significant in case of $p<0.05$.

Discussion

Formation of a functional maternal-fetal system is essential for the progressive functioning of the placenta during placentation with the establishment of maternal-fetal vascular circulation and decrease of pregnancy complications. An important morphological indicator of placental functional status, reflecting the expression of metabolic processes between mother and fetus, is the vascularization of chorionic villi [1]. The development of the functional placental vascular network requires the continuous formation of new blood vessels involving two successive mechanisms: vasculogenesis (formation of vessels from endothelial progenitor cells) and angiogenesis (formation of vessels from pre-existing vessels) [2]. According to the study by Regnault T. R. et al. (2002), defective angiogenesis is often the cause of intrauterine developmental restriction [3] as a result of vascular disturbances [4]. Placental insufficiency related to defective angiogenesis is frequently the cause of severe complications (IUGR, PE, etc.), including placenta accreta [13]. The physiological development of the placental vascular network takes place under the action of various angiogenic factors, the angiopoietin family being one of the most important.

Angiopoietins are a family of extracellular ligands, integral proteins involved in angiogenesis and vascular remodelling. According to the molecular profile, 4 ligands have been elucidated (Ang1, Ang2, Ang3, Ang4), the first 2 and their tyrosine kinase-like receptors having significance in placental vascular morphogenesis (TIE-1 and TIE-2) [5].

Ang1 is a protein made of 498 aminoacids and is having a NH2-terminal coiled domain and a COOH-terminal fibrinogen domain [14], which *in vivo* promotes angiogenesis [15], and *in vitro* is a chemotactic factor for human endothelial cells, not having a cell proliferation effect [14, 16]. Ang1 acts as a paracrine agonist of the Tie2 receptor by phosphorylating and dimerizing the receptor with activation of signaling pathways including the phosphoinositide-3-(PI3)-kinase/Akt pathway and extracellular signal-regulated kinase (ERK) [17]. In this context, angiopoietin-1 represents endogenous ligands of the vascular endothelial-specific tyrosine kinase TIE-2 receptor. It has endothelial and non-endothelial effects and is expressed in the early period of placentation [7].

The Ang1 immunoexpression can be noticed quite early in placentogenesis (week 4) with localization only in syncytiotrophoblast. It is also poorly expressed in the endothelium of blood vessels in immature intermediate chorionic villi, which explains its involvement in vascular promotion and stabilization [7].

The ANG1/ TIE2 signaling pathway promotes endothelial cell survival, endothelial integrity through recruitment and interaction with periendothelial cells, anti-inflammatory/antiapoptotic responses in case of reduced vascular permeability [6, 15] which attributes to it a role in vascular bed formation and stabilization.

In this context, the Ang1 immunoexpression in the germinative site was assessed by immunohistochemical investigation, and the angiogenic profile was described by location and intensity (tab. 2, 3, 4). As a result, the immunoexpression was different both by cellular profile and in relation to the type of early compromised pregnancies, confirmed by statistically significant differences.

Were found some statistically significant correlations between the stromal/mesenchymal cell site and the placental vascularization index (negative in the 6-9 weeks group and positive in the 10-12 weeks group) in the stagnant pregnancies batch. These correlations confirmed the differential angiogenic effect in the formation of the vascular network in the chorionic villous stroma. Thus, differential immunoexpression within groups brings the idea of Ang1 involvement in vascular formation and stabilization during the early period of placentation. This is the case of stagnant pregnancies, in which vascular disruptions are more pronounced and the vascular density is reduced (tab. 4). In order to confirm the given hypothesis, was analyzed the immunoexpression in the research sites vs chorio-villous vascular density. Statistically significant correlations were found in the SP group, in the 10-12 weeks group. The involvement of stromal/mesenchymal cells and Hofbauer cells was noticed in the formation of angiogenic medium. This could be the result of a paracrine effect on the endothelium of blood vessels (tab. 6). The results obtained are in agreement with the authors' data supporting differential cellular immunoexpression of Ang-1 in the placental parenchyma, including the diversity

of the degree of expression, ranging from absent to high degree [7, 18-20].

Conclusions

The placental period is characterized by a weak angiogenic Ang1 differentiated cellular environment in the chorio-villous germinal site in the group of short term compromised pregnancies. The selectively immunoexpressed cellular profile statistically significantly correlates with placental vascular index and chorio-villous vascular density in stagnant pregnancies.

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

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