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An increase of elastic tissue fibers in blood vessel walls of placental stem villi and differences in the thickness of blood vessel walls in third trimester pre-eclampsia pregnancies

Research Article

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Abstract: This study has goals of examining whether pre-eclampsia may lead to an increase of elastic tissue fibers in blood vessel walls of placental stem villi or whether there are differences in the thickness of blood vessel walls within these villi when compared to normotensive pregnant women. Non-infarcted placental tissue samples from 28 participants with uncomplicated pregnancies and 26 patients with pre-eclampsia were obtained. After routine histological procedures, the sections were processed either for conventional Verhoeff staining for the demonstration of elastic fiber system. Paraffine sections from placenta biopsies prepared for light microscopic examination were gathered. In uncomplicated pregnancies, terminal villi blood vessels were observed with no stained elastic tissue fibers in most areas. In the pre-eclampsia pregnancy of human placenta, the elastic fibers significiantly increased in terminal villi blood vessel walls which were dark in color, using Verhoeff's tissue stain, when comparing with the uncomplicated pregnancy group. Our results indicate that an increase of elastic tissue fibers in blood vessels of placental stem villus and terminal villi, and also an increase of wall thickness during pre-eclampsia.

Keywords: *Placenta • Pre-eclampsia • Elastic fibers*

1. Introduction

Pre-eclampsia (PE), traditionally characterized by hypertension and proteinuria in previously normotensive women, remains one of the most leading causes of maternal and fetal mortality in the developed world, and may have an incidence as high as 10% in certain population groups [1]. The etiology of pre-eclampsia/eclampsia still eludes researchers, but most studies indicate that the pathogenesis is likely to be found in the placental bed [2].

So far, contradictory results have been published for the fetal placental blood vessel tree. Wilhelm et al. described the thickness of these blood vessel walls as being enhanced in pre-eclampsia [3]. Umblical arteries, at the fetal side, divide into a number of radially disposed chorionic arteries that branch freely in the chorionic villi. The blood vessels form an extensive arteriocapillary-venous system within the chorionic villi, which bring the

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fetal blood extremely close to the maternal blood. The thin-walled veins that follow the capillaries arteries to the site of attachment of the umblical cord, where they converge to form the umblical vein [4]. In this situation, vessels show a reduction in elastin content due to decreased biosynthesis and accelerated degradation [5,6], sometimes associated with increased migration of smooth muscle cells and vessel thickening [7]. These abnormalities in the umbilical system disturb fetal blood flow and vascular structure. Fetuses from women with pre-eclampsia and with intrauterine growth retardation often suffer from chronic hypoxia, fetal hypertension and raised vascular resistance of the descending aorta [8]. There is also evidence of redistribution of fetal cardiac output, in order to maintain brain oxygen needs [9]. These data suggest the possibility that abnormal haemodynamic environment during pregnancy could alter elastin deposition during fetal development with consequences for the vascular system in adulthood. Since it is known that elastic tissue fibers increase in systemic hypertension, it may be assumed that the enhancement of elastic tissue fibers in placental stem villi during pre-eclampsia may be induced by the hypertension [10]. Nevertheless, the great range of varieties and the differing amounts of elastic fibers in the blood vessels of all investigated placenta was striking [3].

Elastic fibers are essential extracellular matrix macromolecules comprising an elastin core surrounded by a mantle of fibrillin-rich microfibrils. They endow connective tissues such as blood vessels, lungs and skin with the critical properties of elasticity and resilience, but still surprisingly poorly understood. They are important because they endow critical mechanical properties on elastic tissues and regulate cell fate in developing tissues such as blood vessels [11].

Mature elastic fibers and lamellae are comprised of a homogenous core containing a macromolecular polymer called elastin that is assembled along a scaffold of parallel microfibrils. Microfibrils are composed of numerous heterogeneous glycoproteins, such as fibrillin-1 and fibrillin-2, microfibril-associated glycoproteins (MAGP1 and MAGP2) and the latent transforming growth factor β (TGF-β)-binding proteins). Microfibrils also interact with chondroitin sulfate proteoglycans (versican, biglycan, decorin) and other proteins localised to the elastin–microfibril interface or to the cell surface–elastic fibre interface, called elastin microfibril interface-located proteins (EMILINs) and fibulins [11,12]. Elastin is the largest component of elastic fibers, comprising approximately 90%. It is a macropolymer of a precursor protein called tropoelastin, which, in arteries, is synthesized mostly by smooth muscle cells, while adventitial fibroblasts and endothelial cells also display some elastogenic abilities. Tropoelastin is encoded by a single gene localized in human chromosome 7. The modulation of elastin gene expression has not yet been completely elucidated. It has been shown, however, that the gene can be down-regulated by several factors including vitamin D, ascorbic acid, basic fibroblast growth factor, tumor necrosis factor-α and cAMP, as well as up-regulated by insulin-like growth factor, transform transforming growth factor-β (TGF-β) and trivalent iron, among others [11].

Elastic fibres have a short period of active synthesis, restricted to fetal life and the neonatal period. However, it is well established that the development of hypertension is associated with an increase in extracellular matrix (ECM) proteins, including elastin, in the arterial wall. The above-mentioned studies suggest that elastin synthesis can be stimulated by high blood pressure itself. However, there are also some reports in favour of a genetically determined factor, independent of blood pressure, involved in increased elastin deposition [13]. Increased elastin production and accumulation is a rapid and sensitive response to elevated vascular wall stress in both systemic and pulmonary hypertension. While initially protecting the vessel wall, these structural changes may in the longer term result in reinforcement of the hypertensive state and contribute to the persistence of the pathology of hypertension. However, the increase in elastin has been shown to occur in parallel to an increased number of vascular cells, so that total elastin, not elastin content relative to wall mass, is elevated, which suggests an initial cellular defect. Whether this primary cellular alteration is stimulated by an abnormal haemodynamic environment or it is of genetic origin remains to be determined [14]. Genetic defects of elastic fibers, such as supravalvular aortic stenosis, related to an alteration in the elastin gene and defective elastin deposition, are linked to abnormalities in the number of lamellar units. In these cases an increased number of units accompanies the deficiency of elastin [15].

It has been shown that modulation of intracellular iron levels may affect expression of genes that encode extracellular matrix components,including elastic fibers. Patients with β-Thalassaemia, sickle cell anemia and other inherited haemolytic disorders characterized with fluctuating iron levels, also show elastic fibre abnormalities [16].

The study was designed to investigate the histological changes in placentas from pregnancies. The aim of our study was, therefore to examine whether pre-eclampsia may lead to an increase of elastic tissue fibers in blood vessel walls of placental stem villi

and whether there are differences in the thickness of blood vessel walls within these villi when compared to normotensive pregnant women.

2. Material and Methods

2.1. Tissue samples

The study was approved by the institutional review board of the Departments of Obstetrics and Gynecology, Histology and Embryology, Anatomy and Pathology. Non-infarcted placental tissue samples (1X1X1 cm) were obtained from a total of 28 participants with uncomplicated pregnancies (controls:36, and 39-41 weeks of gestation) and from 26 patients with pre-eclampsia (PE: 36, and 39-41 weeks of gestation).

Patients with preexisting arterial hypertension, diabetes mellitus or other diseases were excluded. PE was designed as the presence of blood pressure values of at least 140/90 mm Hg in a previously normotensive woman, measured at least twice with six or more h between the individual measurement and a concomitant significant proteinuria of ≥0.3 g/l per 24 h. After delivery, the placentas were weighed and examined from the maternal aspect to avoid sampling from the areas of obvious infraction. Full-thickness samples were taken from the central part and preparation were performed. Clinical data and characteristics of the study groups are given in table. This definition was given by the National High Pressure Education Program [17].

2.2. Ethics

The local ethics committee gave approval and all participants provided written informed consent.

2.3. Verhoeff staining

The specimens were immersed in Bouin's fixative and after routine histological procedures paraffin sections (5µm) were stained by Verhoeff elastic stain [18].

Procedure:

1.Deparaffinize and hydrate to distilled water.
2.Stain in Verhoeff's elastic stain working solution for 15 minutes.
3.Wash in distilled water.
4.Differentiate in 2% ferric chloride solution.Check microscopically. Elastic fibers are black and sharply fined; the background is gray.
5.Place in 5% sodium thiosulfate solution for 1 minute.
6.Wash in tap water for 5 minutes.
7.Place in distilled water.
8.Differentiate rapidly through 95% ethyl alcohol.
9.Dehydrate in 2 changes xylene.
10.Mount in Permount.

2.4. Statistical analysis

Data are reported as mean ± standard deviation values (DS). Statistical analyses were performed using an unpaired Student's t-test. The data were analyzed using Excel 2000 (Microsoft, USA) and Sigma Plot 2001 (SPSS, USA). We considered $p < 0.05$ to indicate statistical significance.

Table 1. Clinical data of control and pre-eclampsia. Data are given as means ±SD or ratio (*n* = number of investigated placentae, SBP = systolic blood pressure; DBP = diastolic blood pressure).

	Control (n = 28)	PE (n = 26)
Maternal age (y)	22.2 ± 6.2	21.4 ± 5.8
Gestational age (weeks)	39.2 ±1.3	39.6 ±0.9
SBP (mm Hg)	110.8 ± 8.2	155.6 ± 20.2
DBP (mm Hg)	70.15 ± 8.15	90.85 ± 11.2
Proteinury (mg/dl)	43 ± 2	350 ± 3
Birth weight (kg)	3.650.0 ± 342.0	2.850.0 ± 222.0

3. Results

Pregnancy characteristics of each group are presented in Table 1. The uncomplicated pregnancy group and the pre-eclampsia pregnancy group were similar in age and gastational age at delivery. However, the mean arterial pressure, birth weight, and placental weight of preeclamptic women were significantly different than those of the control subjects (P≤0.01).

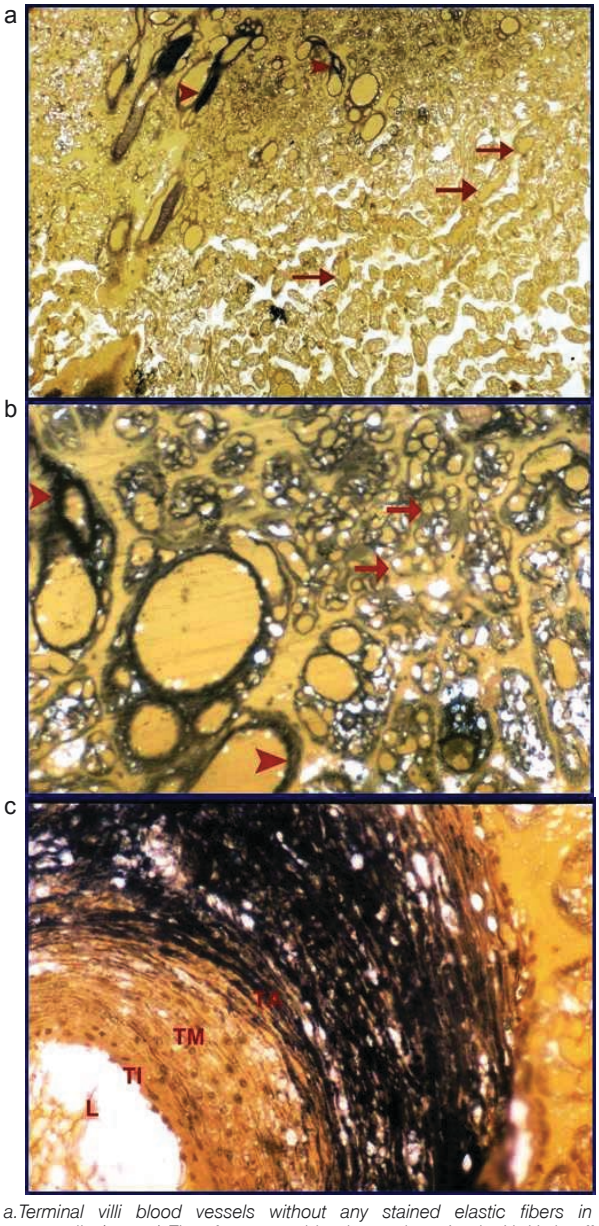
In the uncomplicated pregnancy group, the terminal villi blood vessels which were devoid of elastic tissue fibers showed no staining. Some of the blood vessels were dark with staining Verhoeff's elastic tissue stain (Figure 1a).

In another paraffin section of uncomplicated pregnancy, terminal villi blood vessels observed with no stained elastic tissue fibers in most areas, but in restricted areas elastic fibers were seen in blood vessel walls with Verhoeff's elastic tissue stain (Figure 1b).

In another high magnification section of human placenta of the uncomplicated pregnancy, stem villus blood vessels can be observed. The histologic tunicas of the vessel wall were seen just around the lumen, first the tunica intima, then the media and finally, the adventia. Though, the tunica intima and tunica media of the vessel wall had no staining with Verhoeff's elastic tissue stain but the tunica adventia observed dark containing of elastic tissue fibers (Figure 1c).

In the pre-eclampsia pregnancy of human placenta, the elastic fibers significiantly increased in terminal villi blood vessel walls which were seen dark with Verhoeff's elastic tissue stain when comparing the uncomplicated pregnancy group (Figure 2a).

Figure 1. Paraffine sections of human placentae, uncomplicated pregnancies.



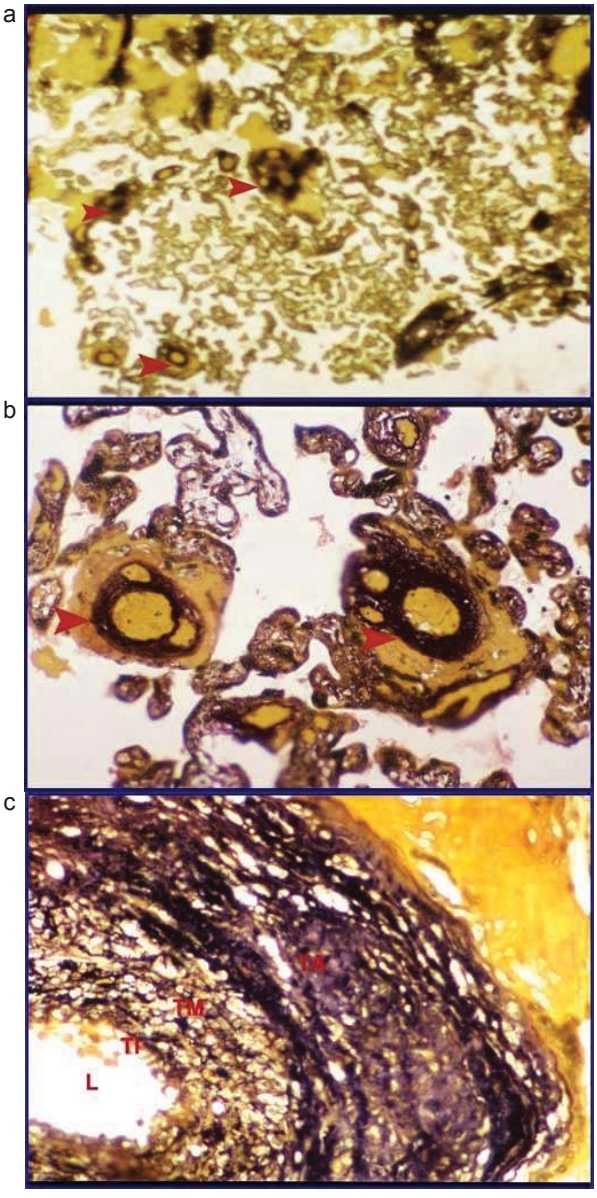
a.Terminal villi blood vessels without any stained elastic fibers in generally (arrows).Therefore some blood vessels stained with Verhoeff (arrowheads)

b. Higher magnification of human placentae, uncomplicated pregnancy. Terminal villi blood vessels without any stained elastic fibers in most areas (arrows) but in restricted areas elastic fibers are seen in blood vessel walls with Verhoeff (arrowheads)

c. Paraffine sections of human placentae, uncomplicated pregnancies. Stem villus blood vessels can be observed with lumen (L), tunica intima (TI), tunica media (TM) and tunica adventia (TA) (Verhoeff staining, original magnification x 16,x 82,x 164)

In the another paraffin section of human placenta of pre-eclampsia group, elastic fibers increased significiantly in terminal villi blood vessel walls and due to an increase of elastic fibers the thickness of vessel wall also observed thicker when compared with the uncomplicated pregnancy group (Figure 2b).

Figure 2. Paraffine sections of human placentae, pre-eclampsia.



a. The increasing of the elastic fibers significantly in terminal villi blood vessel walls (arrowheads)

b. Human placenta of pre-eclampsia.Widespread increase of elastic fibers in terminal villi blood vessel walls resulted in thickness of vessel walls. (arrowheads)

c. Human placenta of pre-eclampsia, elastic fibers increased in tunica media (TM) and tunica adventia (TA) in the blood vessel of stem villi and resulted extreme thickness in vessel wall. Lumen (L), tunica intima (TI) (Verhoeff staining, original magnification x 16,x82,x164)

In the high magnification of the pre-eclampsia group, elastic fibers distributed throughout the blood vessel wall in stem villus. Elastic fibers concentrated in the tunica intima, media and adventia were observed dark with Verhoeff's elastic tissue stain. Accompanying elastic tissue fibers increased the vessel wall of the stem villus was also seen extreme thick (Figure 2c).

4. Discussion

Pre-eclampsia is one of the most threatening diseases for mother and child during pregnancy [3]. Barros et al. researched the presence of elastic fibers by light and electron microscopy in first, second-trimester and term human placentas. Light microscopic analysis revealed elastic fibers in the stroma of main stem villi and in large vessels present in the chorionic plate of term placentas [19].

Over the last decade, there have been considerable interest in the influence of fetal factors in adult disease and in particular, cardiovascular disease. Proponents of the "fetal origins" hypothesis have suggested that cardiovascular health in adult life is associated with nutrition in utero and early childhood. Based on epidemiological studies showing that in adults, the highest blood pressure levels occurred in individuals who had been small in size as infants, Barker et al. proposed that subtle impairment in nutrition during development might predict hypertension and atherosclerosis later in life [20,21]. Among others, a possible mechanism linking reduced fetal growth and raised blood pressure might be changes in vascular structure, including loss of elasticity [22]. The elastic properties of large vessels depend largely on the presence of elastic fibers. Since they are only actively synthesized during embryonic development and early postnatal years [23-26] and they are critical for the mechanical properties of blood vessels, it has been proposed that impaired or defective elastogenesis could be an initiating event in the pathogenesis of hypertension [27,28]. These authors propose that a relative deficiency (or a qualitative defect) in elastin during the period of active elastogenesis, together with the loss and fatigue of elastic fibers during the life of the individual, would gradually reduce the compliance of large vessels and lead to higher pulse pressure in adult life.

Hypertension has also been implicated in arterial wall remodeling. Studies have shown that hypertension is responsible for the increase in arterial wall thickness and changes in the structural composition of the arterial wall. Sustained hypertension leads to structural changes of the arterial wall. These alterations include increases in the degradation and synthesis of collagen and the destruction and reconstruction of elastin fibers, which eventually lead to remodeling of the arterial wall and modifications of its mechanical properties. The mechanism by which hypertension accelerates atherogenesis remains unclear. Tensile stretch on vascular cells may influence cell behavior, such as proliferation, apoptosis, and alterations in gene expression of the ECM [17,29]. However, it is well established that the development of hypertension is associated with an increase in ECM proteins, including elastin, in the arterial wall. This, with few exceptions [30] has been reported in rat with genetic [31], and experimental hypertension [14,32-34].

There is clear evidence for an increased placental oxidative stress in preeclampsia, and although the cause of this still remains uncertain [35]. Recently, Arribas et al. reviewed extensively elastic fibers and vascular structure in hypertension. They presented current knowledge regarding components of elastic fibers and discussed their possible pathomechanistic associations with vascular structural abnormalities and with hypertension and development or progression [17].

Graff *et al.* described the presence of elastic tissue fibers as an essential component of fetal blood vessel walls within trunci and rami chorii in human placental stem villi and umbilical cord vessels [36]. In addidition, Saleh and Dkhıl proposed that ischemic damage of placental tissue caused maldeveloped terminal villi. These findings were consistent with an increase in fetoplacental vascular impedance where absent end-diastolic flow velocity was demonstrated in umbilical artery before delivery [37].

In our study, we showed both a significant increase of elastic fibers in blood vessels of placental stem villi and an increase of elastic-type blood vessel walls during pre-eclampsia. This result was independent of the gestational weeks investigated. When we compared the preeclampsia group with the controls, we found an increase of wall thickness in pre-eclampsia group. Our results were in agreement with the findings of Wilhelm *et al.* [3].

Wilhelm *et al.* described in their recent study that an increase of elastic tissue fibers in blood vessel walls of placental stem villi during pre-eclampsia when compared to uncomplicated pregnancies, they also described in their another study that elastic tissue fibers were decreased during pregnancies complicated by intrauterin growth retardation, independently of the occurrence of concomitant pre-eclampsia when compared to uncomplicated pregnancies [3]. Similarly, Junek *et al.* indicated that PE induced in umblical cord arteries a decrease of elastin concentration accompanied by a thickening of both the intima and the media. Furthermore, they suggested that the absolute elastin content in these vessels was not seem to be significantly changed. PE-associated changes of hemodynamic conditions as fetal hypoxia, fetal hypertension and discontinuous umbilical blood flow e.g. induced by the raised vascular resistance of the descending aorta might be chronic stimuli on the vessel walls in the umbilical cord, leading to a remodeling of the elastic fibre system as described in their study

[7]. All these data suggest the possibility that abnormal haemodynamic environment during pregnanacy could alter elastin deposition during fetal development, with consequences for the vascular system in adulthood.

Since it is known that elastic tissue fibers increase in systemic hypertension, it may be assumed that the enhancement of elastic tissue fibers in placental stem villi during pre-eclampsia may be induced by the hypertension. To get further insight into this assumption, we examined the amount of elastic tissue fibers in stem villus blood vessels of placentae of pregnancies complicated with preeclampsia.It can be assumed that hypertension and hypoxia may lead to a raised fetal vascular wall stress in placental stem villi, thus inducing an increased production of elastic fibers by vascular smooth muscle cells concomitant to their proliferation,in order to protect the vessel walls. In the study of Stenmark *et al.*, they described that morphometrical examination of the lung arteries had showed markedly thickened adventitia with cellular proliferation and collagen and elastin deposition. They also saw increased medial thickness and distal muscularization of the pulmonary arteries associated with decreased luminal diameter. Stenmark et al. described that the rapid development of severe pulmonary hypertension and poor responsiveness to O2 was associated with increased arterial wall thickness, particularly involving the adventitia [38].

At the same time, Stenmark et al. discussed that increased proliferation and elastin secretion of the smooth muscle cells could finally lead to the thicker vessel wall that they found in their study, and may protect the whole placental blood vessel tree against local stres. Also our findings about the thickennig of the blood vessel walls were also similar with the study of Stenmark *et al.* [38]. Some investigations reported a thickening of blood vessel walls during systemic hypertension, which was discussed as hypertrophy or hyperplasia. Similar changes of the structure of fetal stem arteries from placentae in 'toxemia' (PE, hypertension and chronic renal disease) are published by the group of Las Heras. In studies based on third order fetal arteries, Las Heras et al. found an endothelial proliferation and a proliferation of subendothelial cells and smooth muscle cells in the media of stem villi arteries of 'toxemic' placentae [39]. By transmission electron microscopy Las Heras and Haust explained the proliferation of smooth muscle cells in the media of stem villi arteries in toxemic placentae as an absolute increase in numbers of arterial smooth muscle cells and a considerable proliferation of abasement membrane-like substance [39,40]. In addition, there was a definite increase of collagen fibrils in the interstitial spaces. From the present study it is obvious that the general distribution pattern of elastic fibers within the stem villus blood vessel walls does not depend on the status of gestation, *i.e*. uncomplicated or pathological. However, a significant increase of elastic fibers distributed throughout the blood vessel wall or in segments was detected in cases of PE when compared to uncomplicated pregnancy.Possibly, elastic fibers being distributed in segments may represent early stages of developing elastic-type blood vessels following rising blood pressure.

In conclusion, most of the studies to date have focused on alterations in vascular collagen and, in fact, it is now established that increases in collagen content are key to the vascular mechanical alterations in preeclampsia. It is surprising, however, that elastic fibers, being the main components of conduit arteries and largely responsible for vascular elasticity, have not been thoroughly studied. These histological findings of elastic fibers in third trimester pre-eclampsia pregnancies has likely contributed to this lack of information. An increase of elastic fibers in blood vessels of placental stem villi, an increase of elastic-type blood vessel wall segments and an increase of wall thickness during pre-eclampsia were clearly showed histologically in preparations. We suggest that these alterations may be related to placental hypoxia and raised maternal and/or fetal blood pressure and we assumed that the increase of elastic fibers and wall thickness could be mechanisms of the stem villus blood vessels to protect the vessel wall and maintain an adequate fetal blood.

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