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RESEARCH ARTICLE

Microscopic Manifestations of Maternal Diabetes in Placental Structure

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ABSTRACT

The human placenta is crucial for prenatal development and good pregnancy outcome. Maternal diabetic disorder influences normal intrauterine development of individual and expresses itself in the placenta. Due to the progress in diagnostic methods and methods of metabolic compensation the macroscopic signs associated with maternal diabetes, e.g., placentomegaly, occur nowadays rarely. In the microscopic picture of placenta there are no structural differences specific for maternal diabetes. However, the application of quantitative morphological methods has revealed some differences of normal and diabetic placenta. Stereological studies have shown significantly higher total volume of peripheral villi that distorts shapes and dimensions of pores in the intervillous space, and larger surface areas of peripheral villi and villous capillaries. Methods of confocal microscopy and 3D reconstruction gave the evidence that the fetus reacts on the hypoxia in maternal diabetic environment by enhanced angiogenesis and branching of villous capillaries. The enhanced tissue volumes are conditioned by higher mitotic activity. However, it decreases the proliferative potential of cells taking part in the enlargement of key structures for maternofetal transport at the end of pregnancy. Syncytiotrophoblast produces many factors playing important roles in maternal and fetal regulation and in the placenta itself. Quantitative methods of catalytic histochemistry and immunocytochemistry pointed at the role of maternal diabetes in decreased synthesis of some of those factors (e.g., alkaline phosphatase, SP1 glycoprotein). The presented data show that the quantitative morphological methods contribute to better understanding of the influence of maternal diabetes on fetoplacental unit.

1. Introduction

The placenta is temporary organ ensuring normal intrauterine development of the individual. Concurrently with the fetus, the placenta increases its volume and weight to be properly equipped for the meeting all fetal requirements. The bidirectional transport of gases, ions, nutrients, and metabolites covers fetal requirements. Obviously, a portion of substances taken from the maternal blood serves for placental growth and synthesis of molecules controlling functional relationships of maternal and fetal organism and placenta itself, e.g., steroid and glycoprotein hormones, growth factors, cytokines, pregnancy associated plasma proteins, releasing factors and so on.

The relatively short period of placental existence covers its growth, structural development, and functional maturation. Our chance to study the placenta in various stages of normal as well as pathological pregnancy is limited by many ethical and technical reasons. Certainly, biochemical analysis of maternal blood, the ultrasound examination, etc., give valuable information. Nevertheless, under the circumstance, the examination of term placenta using different morphological methods gives an opportunity to obtain a lot of complex data. This text is a summary of relevant data comparing placental structure in normal pregnancies and pregnancies complicated by maternal diabetes mellitus using methods of quantitative morphology. The possible functional consequences of structural differences are presented.

The human hemochorial placenta consists of two parts, one of the maternal origin, *pars materna*, the other of fetal origin, *pars fetalis*. The maternal part is derived from uterine mucosa changed by decidua reaction, *decidua basalis*. It forms a bed for fetal part derived from chorion. The part of chorion adjacent to *decidua basalis*, i.e., *chorion frondosum* develops into chorionic plate and its tree-like projections, chorionic villi. Some villi fix the fetal part to the maternal one in the form of anchoring villi, the free villi are suspended in the maternal blood circulating in the intervillous space. The chorionic plate and chorionic villi as well as the surface of maternal part are covered with trophoblast. In villi, the trophoblast surrounds villous core of connective tissue containing villous vessels that belong to the fetal vasculature. The transport between mother and fetus takes place at the level of capillaries of the thinnest villous branches, terminal villi. Both the essential separation of maternal blood from the fetal one and the bidirectional transport are performed by trophoblast and capillary wall.

Pregnancy complications are the major problem of prenatal medicine as they threaten both the mother and fetoplacental unit. Maternal diabetes represents one of most frequent one, and it is beyond the scope of this paper to discuss the influence of higher maternal age or lifestyle in developed countries on its frequency. In any case, all forms of glucose metabolism disorders of maternal organism, i.e., diabetes type I, gestational diabetes, and diabetes type II generate the environment potentially affecting fetal development and causing structural and functional disorders of placenta. In pregnancies complicated by diabetes type I only the introduction of insulin allowed to achieve a viable newborn. Nevertheless, the uncontrolled diabetes may affect the development in embryonal and fetal period of pregnancy resulting in congenital malformations. The newborns of diabetic mothers were also macrosomic and suffered from birth injuries and respiratory distress. The hypocalcemia, hypoglycemia, polycythemia, and hyperbilirubinemia were common metabolic complications in the perinatal period. The placenta in those pregnancies was usually bulky and edematous.

The influence of maternal diabetes on fetus is mediated and modified by placenta interposed between mother and fetus, and the placental structure necessarily reflects fluctuations of metabolic control in maternal organism. Only the progress in the management of metabolic control in preconceptional period and throughout pregnancy resulted in reduction of the impact of maternal diabetes on the placenta, fetus, and newborn [1,2].

2. Quantitative morphology

Methods of morphometry and stereology using various types of microscopes allowed quantification of structural differences in normal and diabetic placentas for the whole organ. Diabetic placentas taken into those studies were usually classified according to the White´s classification that includes diabetic mothers according to the age of first appearance, duration, and severity of their diabetes into classes A, B, C, D, F, R. For the better understanding it is necessary to mention that class A represents a disorder of glucose metabolism with the onset during pregnancy that is not insulin-dependent and disappears after the birth, other classes belong to the insulin-dependent maternal diabetes of various severity.

Two papers have shown differences in the development and arrangement of placental villi. The study performed by scanning electron microscopy revealed hyper- and hyporamification of terminal villi in diabetic placentas[3]. In the

framework of normal placental cotyledon shorter villi were found in its center and longer villi in its periphery. This consistent organization was disrupted in diabetic placentas and villi were found more branched [4].

Following quantitative morphological studies compared control placentas with placentas from well controlled maternal diabetes at term. In class A, B and C, placentas were heavier, but that difference was not significant. On the other hand, the volume of peripheral villi as well as surface areas of peripheral villi and capillary surface areas were significantly higher [5-7]. Moreover, the enlargement of the villous surface demonstrates itself also at the level of the apical membrane of syncytiotrophoblast. The microvillous surface enlargement factor and the microvillous surface density were found significantly higher in diabetic placentas [8].

The comparison of control placentas with placentas from pregnancies with well controlled diabetes grouped in ABC and DFR groups demonstrated higher volume, surface area and length of villi in ABC group than in controls, and lower volume, surface area and length of villi in DFR group compared with ABC group. The same character of differences was found in the volume of villi, trophoblast, syncytiotrophoblast and stroma. Furthermore, the expansion of the villous component was accompanied with the expansion of the intervillous space (IVS) during pregnancy. The comparison of normal and diabetic placentas showed higher volume of IVS in ABC vs. control group, and higher volume of IVS in DFR vs. ABC group. Only capillary volume and villous capillarization (= surface area of capillaries/surface area of villi) were ascertained higher in all diabetic groups [9-12]. The length, volume, surface area and capillary diameter were reported greater in diabetic placentas, however, those parameters were greater in placentas of male fetuses, thus they are influenced also by the sex of newborn [13]. No differences of total capillary volume and capillary surface area between control and diabetic placentas were found by other authors [14, 15]. On the other hand, the greater placental capillary volume, length, and surface area in type 1 (insulin-dependent) and type 2 (non-insulin-dependent) maternal diabetes were reported elsewhere[16].

Structural alterations (expanded villous volumes and surface areas) found in diabetic placentas indicate disordered dimensions and shapes of pores of the IVS (Figure 1). As hemodynamic properties of pores in the IVS may play an important role in the transport and exchange between mother and fetus, then maternal diabetes may also alter IVS blood circulation. However, to study rheological properties of such porous medium is still more difficult than to study a system of tubes of vascular bed, and methods of the mathematical modeling and simulation of hemodynamics are applied [17-19].

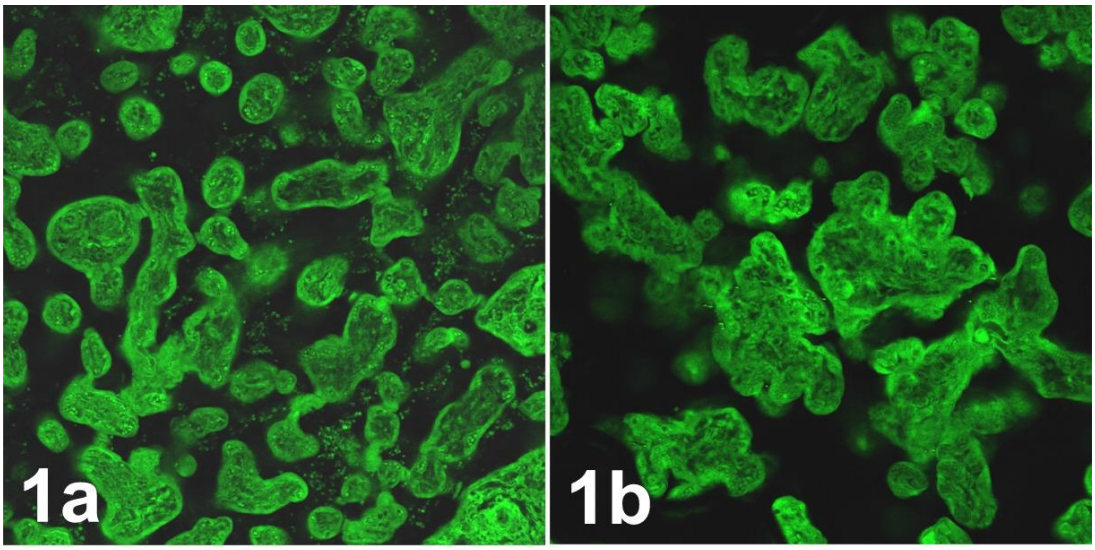

Figure 1: *The shape of normal villi (1a) and pathological villi in diabetic placenta (1b) determines differences in dimensions and shapes of intervillous pores. Pictures taken by a confocal laser scanning microscope, objective magnification 10x.*

3. Villous vascularization

In the last trimester of pregnancy, the immense development of terminal villi and its capillary bed meets rapidly growing requirements of fetal nutrition. Finally, the terminal villi take about 39% of the total villous volume and about 46% of the total villous surface area [2], and the estimation of placental capillary bed is 550 km of length and 15 m^2 of the surface area [20]. In terminal villi, capillaries are in a very tight relationship to syncytiotrophoblast, frequently to its thin anuclear parts. Those areas called vasculosyncytial membranes are taken as sites of preferential transport (Figure2).

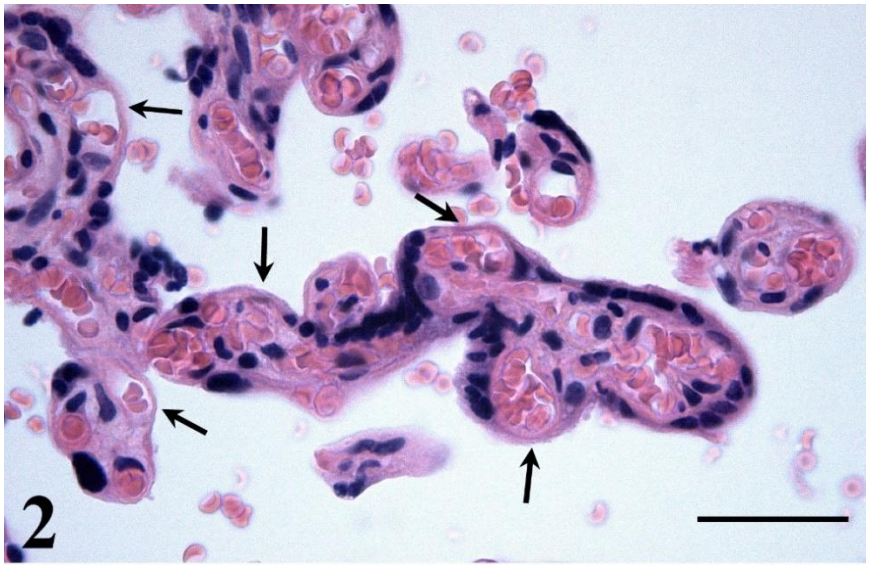

Figure 2: *Terminal villi in the placenta at term. Anuclear parts of syncytiotrophoblast and capillary wall form vasculosyncytial membranes (arrows). Bar = 50 μm.*

The villous capillaries belong to the fetal circulatory system and their density and spatial arrangement determine the effectiveness of the transport of nutrients to fetal tissues. Massive angiogenesis accompanying the development of terminal villi undergoes molecular regulation [21-23] that reacts on changes in the intrauterine environment, e.g., the decreased availability of oxygen. It may be caused among others by the alteration of glucose metabolism in maternal diabetes. Despite the consistent metabolic control applied recently, the intermittent excess of glucose load may cause transient hypoxia and stimulate placental angiogenesis resulting in higher capillarization of terminal placental villi [9-13, 16]. The studies on topology of villous capillaries performed by methods of confocal microscopy and 3-dimensional reconstruction showed villous capillaries of straight or moderately waved course localized commonly in tight relationship to syncytiotrophoblast. The most frequent types of the capillary bed in control placentas consisted either of a simple loop or three longitudinally oriented capillary segments, the capillary segments interconnecting those longitudinally oriented capillaries were also found. However, the quantitative comparison of villous capillary bed with diabetic placentas discovered enhanced angiogenesis in the form of the enhanced capillary branching. Moreover, the enhanced capillary growth, either longitudinal or branching, elicited the development of new branches of terminal villi [24,25]. There is no doubt that it implies enhanced cell proliferation in both the trophoblast and capillary wall.

The enlargement of capillary bed requires the execution of endothelial proliferation, differentiation, and migration allowed by disintegration of endothelial basement membrane, and the recruitment of pericytes to stabilize newly formed part of capillary wall or capillary branch.

The available data refer to the differences in the structure of capillary wall in control and diabetic placentas driving the increase of the total capillary length and surface area. Placental continuous capillaries have tight and adherent junctions between non-fenestrated endothelial cells. As revealed by immunocytochemical method, the immunofluorescence of occludin, zonula occludens 1 protein, cadherin, and β-catenin is reduced in diabetic placenta. It makes endothelial cells freer and suitable for proliferation [26, 27], but this feature also allows blood cells to enter easily extravascular location (Figure 3).

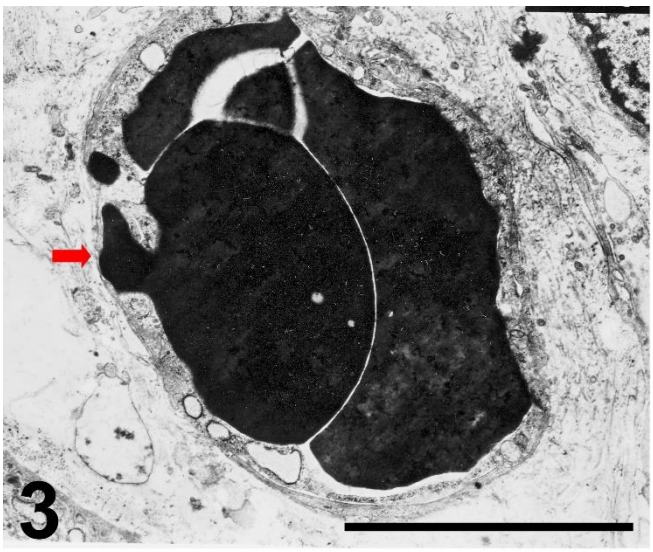

Figure 3: *The fetal erythrocyte leaves the capillary via a gap in the wall (arrow). Bar = 5 µm.*

The capillary endothelium rests on the basal lamina that is locally disintegrated during endothelial division, and finally reconstructed. The thickening of basal laminas is a typical structural feature of diabetes, and it is here and there mentioned also in placenta. However, the measurements showed thinner basal lamina of capillaries in diabetic placentas [28, 29]. Taking into consideration the time necessary for the formation of new spots of the basal lamina after the endothelial cell division, the possible explanation of such difference may be a consequence of higher proportion of newly formed younger capillaries.

During the differentiation a transient cytoskeletal protein nestin occurs in both the endothelial cells and pericytes. The nestin immunolocalization labels the hot spots of villous angiogenesis, i.e., nestin-positive endothelium beside quiescent pericytes and nestin-negative endothelium surrounded with nestin-positive pericyte bodies or projections (Figure 4). The higher proportion of nestin-labeled parts of capillaries found in diabetic placentas indicates more active capillary elongation and branching [30].

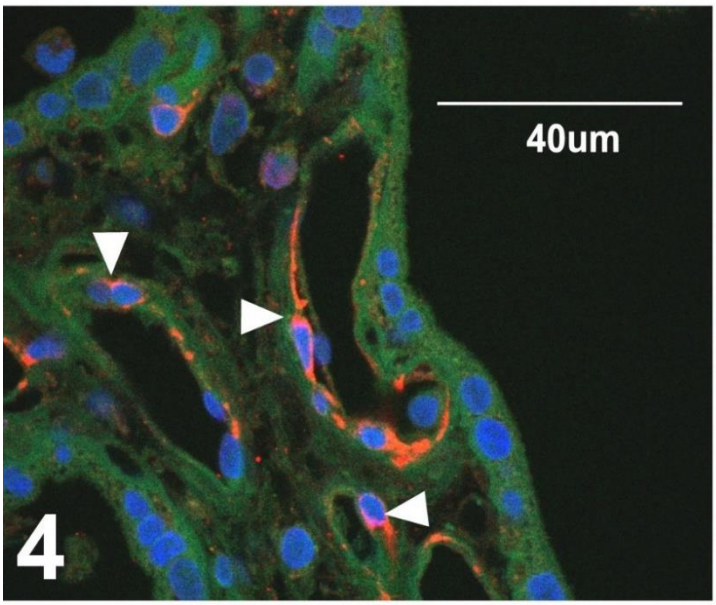

Figure 4: *The nestin immunocytochemistry (red) demonstrates differentiating parts of capillary wall in placental villus.*

The extent of pericyte coverage of capillaries may be indicative for placental hypoxia. The lower pericyte coverage, and thus a decrease of thickness of the barrier separating maternal and fetal blood, was found in placentas of mothers living in high altitude [31]. Similar study performed on diabetic placentas has not demonstrated differences from control placentas [32]. We judge that the lower pericyte coverage is an adaptation to continuously low oxygen pressure in high altitude, and that the intermittent hypoxia taking place in diabetes, on the other hand, does not require such type of compensation.

We believe that the enhanced total volume and surface area of placental villi and their capillaries manifested also by their higher branching in diabetic placenta is a compensation of hypoxia of episodic character, and that compensation is conditioned no doubt by increased cell proliferation.

4. Proliferative potential of placenta at term

Although the placenta is a temporary organ in the life of individual, its full function is required until the birth is completed. Results of the study on proliferative potential using Ki67 as a marker have shown Ki67-labelled cytotrophoblast, cells of capillary wall as well as cells of the villous stroma in placenta at term [33], (Figure 5). It is evident that the ability to proliferate continues in placenta at the end of pregnancy, and points at the potential to develop new villi. In placentas from pregnancies complicated by maternal diabetes the higher H^{3}-thymidine incorporation was found in cells of villous stroma [34] and in endothelial cells [35].

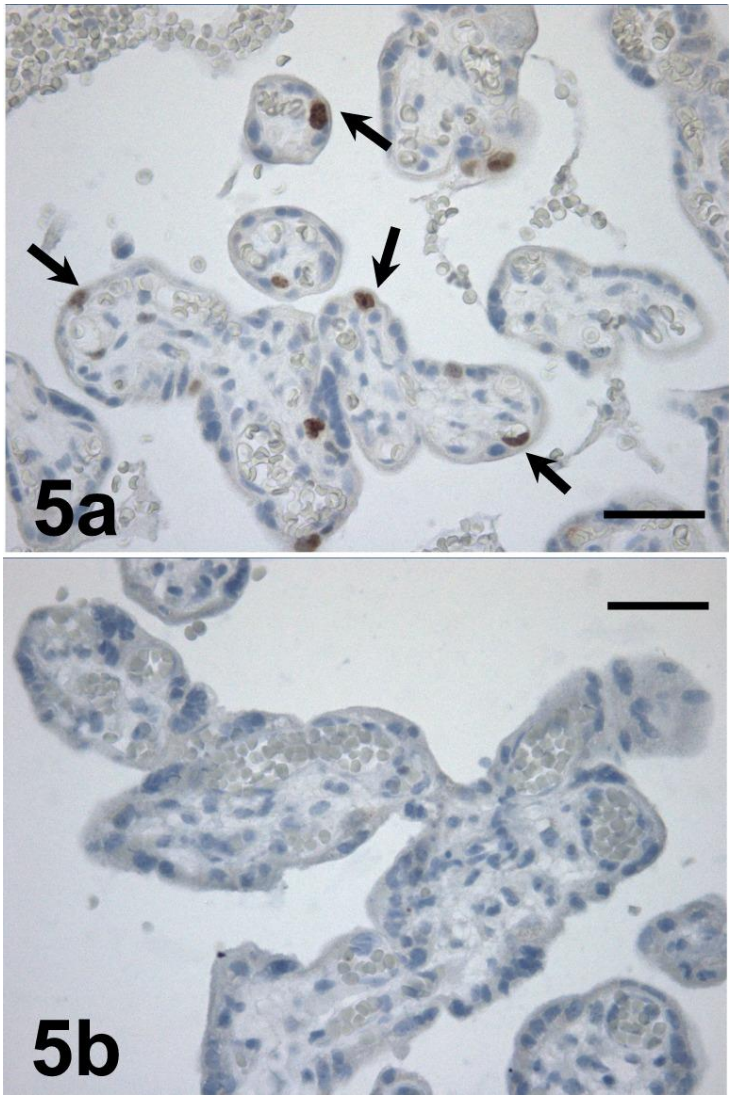

Figure 5: *The Ki67-labelled cells (arrows) demonstrate proliferative potential of cells in normal placenta (5a) whereas Ki67-labelled cells nearly lack in diabetic placenta (5b). Bar = 50µm.*

Our analysis performed on placentas from pregnancies complicated by insulin-dependent and gestational diabetes shows decreased numbers of Ki67-labelled cytotrophoblastic cells and cells of the vascular wall [36, 37]. Those results indicate that the ability to enlarge the areas of key transport structures, i.e., syncytiotrophoblast and capillary wall is probably at least in part exhausted by enhanced cell proliferation during pregnancy (Table 1).

During their life, normal cells undergo limited number of mitotic divisions conditioned by chromosomal replication. It is regulated among others by the length of chromosomal telomeres and every cell division shortens the telomere length. The consequences of telomere shortening may be cell senescence, genome instability, and apoptosis. Studies dealing with placenta have shown the decrease of telomere length during pregnancy, but there is no unambiguous information on the influence of maternal diabetes. It seems that the methods applied there and showing the mean length of telomeres were not adequately sensitive, and that the methods quantifying very short telomeres may contribute to the elucidation of placental senescence in normal and diabetic pregnancy [38 - 40]. Nevertheless, the possible more rapid telomere shortening could have depleting influence on the placental proliferative ability in maternal diabetes.

Table 1: *Proliferative potential in normal term placentas and placentas from pregnancies complicated by gestational (GDM) and insulin-dependent diabetes (IDDM) was expressed as mean number of Ki67-labelled nuclei per square millimeter of villous cross section. In stem villi, the differences relate mainly to capillaries functioning as vasa vasorum of large villous vessels.*

*$p < 0.05$; **$p < 0.01$

GROUP (number of placentas)	**IDDM (16)**	**GDM (13)**	**Control (9)**
STEM VILLI			
Cytotrophoblast	6.21 ± 5.36	6.77 ± 5.27	7.60 ± 5.79
Stroma	5.11 ± 6.51	1.68 ±1.35	2.91 ± 2.47
Vascular wall	1.76 ± 1.94*	1.51 ± 1.53**	3.71 ± 2.07
INTERMEDIATE VILLI			
Cytotrophoblast	9.46 ± 6.12*	10.93 ± 6.42*	18.262 ± 7.741
Stroma	5.43 ± 6.16	1.65 ± 1.13	3.10 ± 4.30
Vascular wall	1.42 ± 1.54	2.07 ± 2.03	4.30 ± 5.51
TERMINAL VILLI			
Cytotrophoblast	18.18 ± 11.90*	17.67 ± 9.01*	29.47 ± 15.87
Stroma	8.94 ± 9.78	1.57 ± 1.02	5.42 ± 7.17
Vascular wall	4.48 ± 3.28*	7.39 ± 4.26	9.82 ± 4.46

5. Synthesis in the syncytiotrophoblast

The change of the maternal environment may elicit a response also in the trophoblast. There are findings of increased number of cytotrophoblastic cells and focal necrosis of syncytiotrophoblast mentioned e.g., in [1], but no reliable quantitative data are available.

Nevertheless, syncytiotrophoblast is a site of synthesis of many factors important for normal course of pregnancy, and maternal diabetes may influence that function. Unfortunately, morphological studies are sparse. The influence of diabetes on distribution of some placental proteins is demonstrated in the study realized on placentas of different White´s classes. The evaluation of immunocytochemical detection was performed by independent observers for β-subunit of human chorionic gonadotropin (βHCG), placental alkaline phosphatase (PLAP), pregnancy specific β-1-glycoprotein (SP1) and human placental lactogen (HPL). The correlation between villous immaturity and weaker staining pattern of PLAP, SP1, and HPL in diabetic placentas found there shows that maternal diabetes affects also synthetic function of syncytiotrophoblast [41].Studies on the quantitative comparison of the catalytic activity of placental alkaline phosphatase and Na^+,K^+-ATPase in syncytiotrophoblast have shown significantly lower mean optical densities of reaction product in placentas from pregnancies complicated by insulin-dependent diabetes than in control placentas [42, 43]. As for the alkaline phosphatase, its location on apical microvillous membrane (and basal cell membrane and coated vesicles -see Figure 6) is like to other absorptive cells (e.g., epithelium of proximal kidney tubules, enterocytes). Although its function is not fully understood, there are data suggesting its role in the placental lipid transport [44]. It is self-evident that the synthesis of all factors in syncytiotrophoblast as well as other placental

functions require a lot of energy, and mitochondria should keep up with those requirements by production of appropriate amount of ATP. The decreased activities of placental mitochondrial respiratory chain enzymes in women with pre-existing obesity and diabetes may negatively influence all placental function and thus fetal growth and development [45].

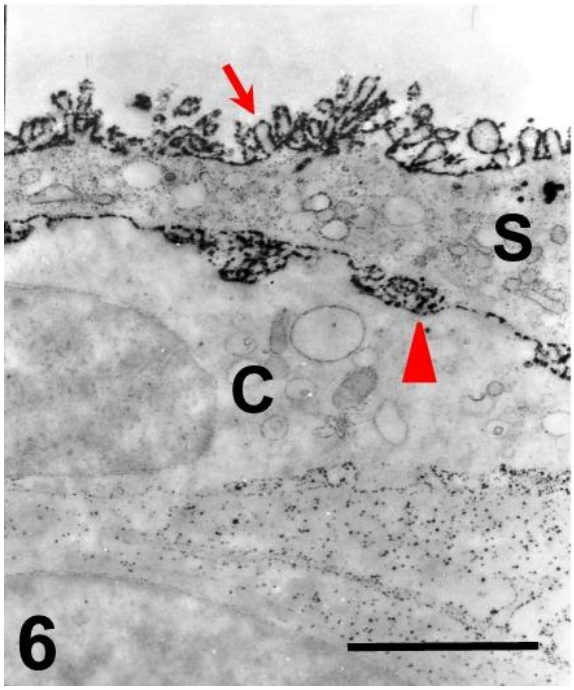

Figure 6: The electron micrograph shows the alkaline phosphatase activity on microvillous (arrow) and basal cell membrane (arrowhead) of villous syncytiotrophoblast (S). C = cytotrophoblast. Bar = 2 μm.

6. Conclusion

The optimal fetal growth and development is conditioned by adequate placental structure and function. The development of individual in the milieu of diabetic mother is characterized by higher glucose supply that is not accompanied with corresponding oxygen supply. Those metabolic conditions elicit compensatory reactions of fetus demonstrated in the placental structure, mainly in peripheral parts of villous trees. The enhanced villous angiogenesis and enlargement of the area of syncytiotrophoblast should provide more oxygen, but it is contradictory because the amount of transported glucose is enhanced as well. The rapid development of villi also produces larger villi of unusual shape that distort dimensions and shape of intervillous pores, and thus the blood circulation in the intervillous space. The impaired function of placental mitochondria turns the attention to the placental metabolism. The consequence of lower energy supply may be the lower synthetic activity of syncytiotrophoblast, however it seems that the amount of synthetized substances may be compensated by increased development of villi. As shown in the study on proliferative potential, the rapid growth of both structures executing placental transport leads to certain exhaustion of placental reserve to enlarge their areas at the final period of pregnancy. It may be related to placental insufficiency and perinatal morbidity and mortality.

The structure of placenta in maternal diabetes displays pathological changes that mirror the effect of therapy during pregnancy. The presented data turn the attention to the fact that the imbalance of oxygen and glucose supplied to the fetus manifests itself in different features showing the fetal reaction, e.g., enhanced villous growth and angiogenesis leading to decreased proliferative potential, as well as the reaction of placenta itself, e.g., decreased synthetic activity and disturbed mitochondrial function. The strong metabolic control may adjust that imbalance and thus to improve placental morphology. Further study is needed for complete understanding of structural changes, distorted regulation processes in fetoplacental unit and possible epigenetic effects on the offspring.

7. Acknowledgements

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8. Conflict of interest

The author declares no conflict of interest.

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