Institute of Experimental Morphology and Anthropology

Acta morphologica et anthropologica, 5
Sofia•2000

Intermediate filament proteins in cells of the human full term placenta

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The expression of intermediate filament proteins such as cytokeratin, vimentin, desmin and GFAP was studied on paraffin sections of full term placenta. Some cells of amniotic epithelium were both cytokeratin and desminpositive. However, the reaction was not very intensive. Positivity to all the antibodies applied in the placenta was expressed by the cytotrophoblastic cells of the chorionic plate only, while the cytotrophoblast of the chorionic trunks and their branches were only cytokeratin and desmin-positive. The syncytiotrophoblast demonstrated a strong expression only to cytokeratins. The endothelium of all blood vessels was stained only for vimentin. Besides, most vascular myocytes and stromal cella were vimentin-positive. Desmin positivity in large vessel walls was mostly expressed in the outer media layers. Based on these data some conclusions on the differentiation and function of single placental cells and their possible interactions influenced by different stimuli are discussed.

Key words: human placenta, intermediate filaments, cytokeratin, desmin, vimentin.

Intermediate filaments (IF) are essential fibrillar components of the cytoskeleton. They are of central importance for cell form and integrity [10, 25, 40, 44]. In the recent 10-15 years, the interest in these structures increased considerably mainly because of the fact that knowledge about their chemical nature and distribution became very important for the cellular differentiation and typing, as well as for the diagnostic pathology and histogenesis of neoplasms [1, 26, 29, 40, 41]. A crucial contribution was also provided by the rapid development of immunohistochemistry.

The expression of IF proteins in human placenta was first observed in cells isolated from amniotic fluid [27, 38, 43, 50]. Numerous immunohistochemical investigations in different placental parts at full term pregnancy, as well as during development have been accumulated [2, 20, 21, 22, 47]. It has to be noted that most of them aimed at presenting some IF proteins in selected normal and in pathologically altered cells [18, 23, 51, 52]. This resulted in certain controversies and doubts whether and to what extent the theory of origin as seen by Osborn et al. [39] can be attributed to the palcenta at all [21].

Therefore we decided to perform immunohistochemical investigation on the expression patterns of the main IF proteins such as cytokeratin, vimentin, desmin, and

glial fibrillary acidic protein (GFAP) in all cells located within the human full term placenta. We hope to be able to provide new evidence in an attempt to contribute to the clarification of certain issues of the nature, differentiation and function of placental cells.

Material and Methods

The study covered the amnionic epithelium, free cytotrophoblast cells of the chorion plate and all the cells (vascular wall, stroma and cytotrophoblast cells, as well as syncytiotrophoblast) of the villous tree from 25 human full term placentas after normal pregnancy. The expression of IF proteins such as cytokeratin, vimentin, desmin, and GFAP (Table 1) was demonstrated on formaldehyde-fixed paraffin sections by

T a b l e 1. List of antibodies used in the study

Antibody	Kind	Manufacturer	Peculiarities
Vimentin	1. monoclonal	Zymed (USA)	both antibodies were tested on the same sections
	2. monoclonal	Boehringer (Germany)	identical results were obtained
Desmin	monoclonal	Zymed (USA)	it was tested on human blood vessels
GFAP	polyclonal	Zymed (USA)	it was tested on spinal cord sections
Cytokeratin PAN	polyclonal	Zymed (USA)	it binds to cytokeratin polypeptides 4, 5, 6, 7, 8, 10, 13, and 18

the streptavidin-biotin immunoperoxidase method [17]. ZYMED Histostain SP (USA) kit was used. Selected sections were poststained with Meyer's haematoxillin. Controls: negative controls were made with the omission of the primary antibody and with NGS, positive controls were incubated with antisera to CD31 to label selectively stained endothelial cells. Observations were made by Zetopan photomicroscope (Reichert, Austria).

Results

The cells of the amnionic epithelium facing the amniotic fluid demonstrate cytokeratin immunopositivity (Fig. 1—*a*). However, this reaction was not strong. It was irregularly distributed in epithelial cells; there were also cells which remained unstained. We counted only the strongly positive amniocytes on numerous sections which were slightly above 50-55% of all. The (PAN) Cytokeratin antibody used in our study is shown to react with broad spectrum of cytokeratins — 4, 5, 6, 8, 10, 13, and 18. The reaction product was fine-granular and was located mainly laterobasally in the cell. Another finding was the presence of amniocytes showing moderate desmin immunopositivity (Fig. 1-*b*) with intracellular distribution similar to that of cytokeratin. However, less than 40% of all amniocytes were stained.

The trophoblastic cells located free — as single cells, or in groups within the chorionic plate (cytotrophoblast of the chorionic plate) displayed a strong immunopositivity to cytokeratin (Fig. 2 — *a*). The reaction product was fine granular in the immediate vicinity of the nucleus. The Langhans' cells of the villous tree were also cytokeratin-positive (Fig. 3). The trophoblastic cells of the chorionic plate reacted also strongly to desmin and vimentin (Fig. 2 — *b, c*), and the reaction could be de-

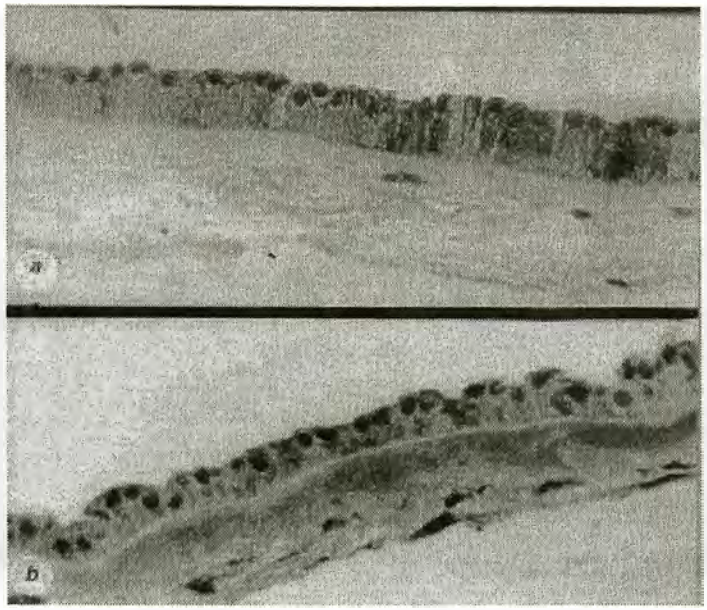

Fig. 1. Amnionic epithelium: cytokeratin (*a*) and desmin-positive (*b*) cells Poststaining with Mayer's haematoxillin (×200)

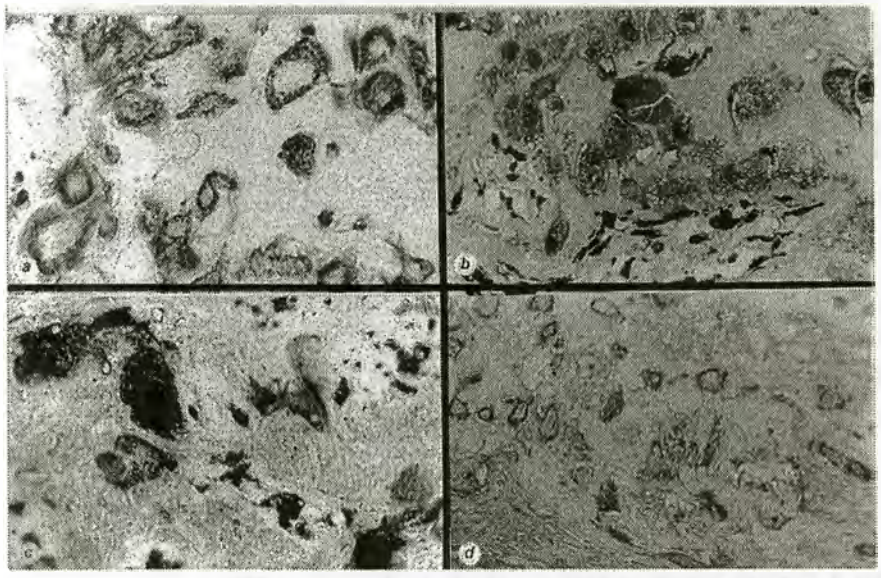

Fig. 2. IF protein expression in cytotrophoblastic cells of the chorionic plate *a* — cytokeratin; *b* — desmin; *c* — vimentin; *d* — GFAP (×300)

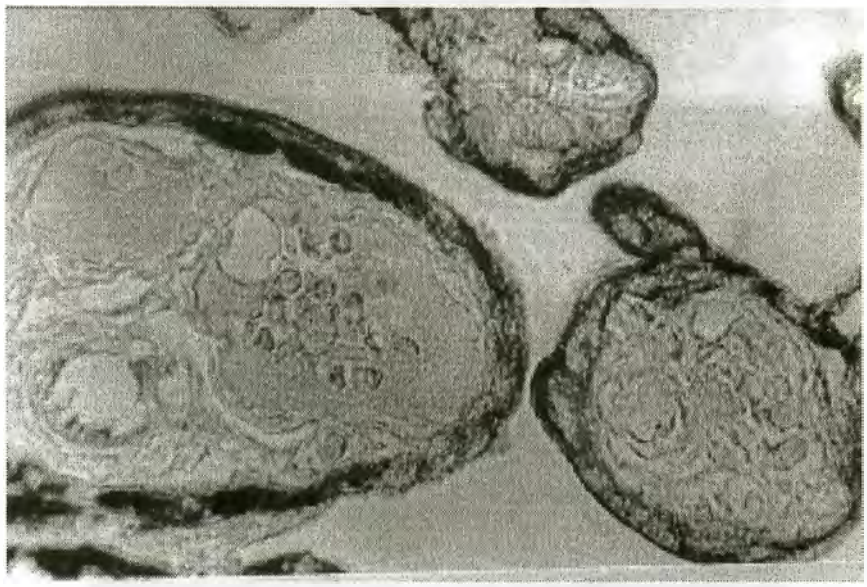

Fig. 3. Cytokeratin immunopositivity in terminal villi (×400)

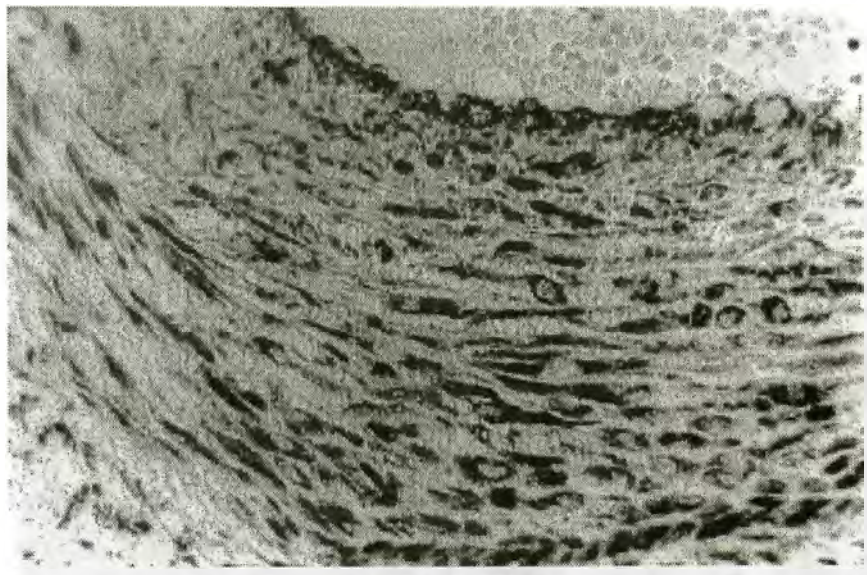

Fig. 4. Vimentin immunopositivity in a chorionic-plate artery (×200)

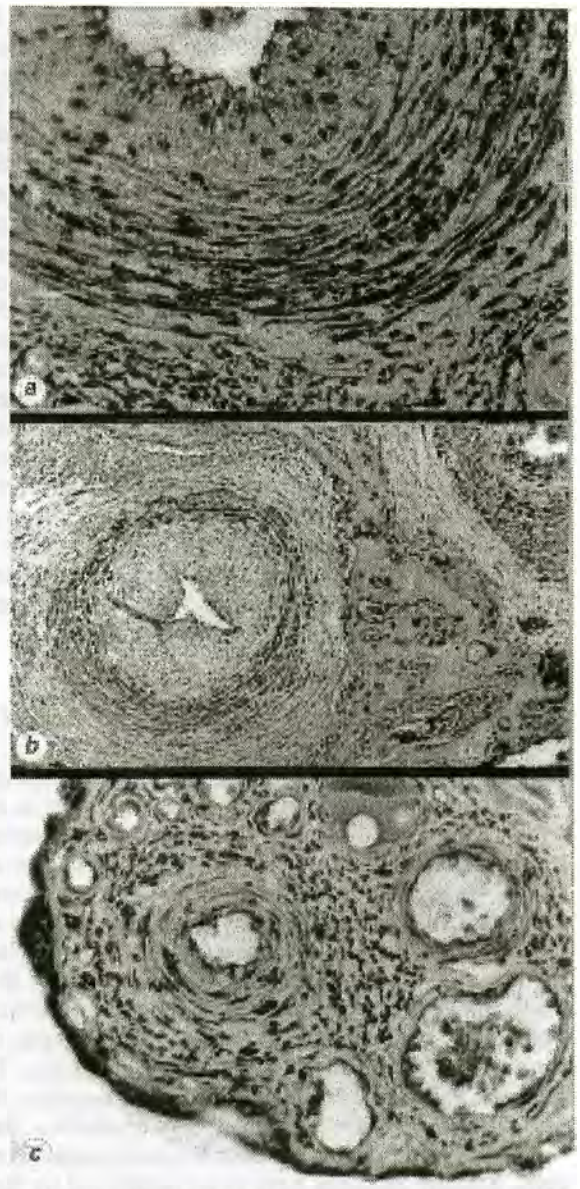

Fig. 5. Desmin expression in differrent placental parts
a — cross section of a villous-trunk artery: intensively stained myocytes of the external media; desminpositive stromal cells (×200); *b* — small-sized chorionic-plate artery and vein; desmin-positive myocytes (×100); *c* — cross-section of primary-villous-trunk branchings (×200). Poststaining with Mayer's haematoxillin

tected in the cellular matrix. GFAP was detected also only in these cells, although the staining was less intensive (Fig. 2 — *d*). Cytotrophoblasts of other localizations such as those in the villi remained GFAP-negative.

The syncytiotrophoblast forming an integral cytoplasmatic cover on the surface of all villi and partially also on the chorionic plate facing the intervillous space reacted strongly to cytokeratin (Fig. 3). It was, however, completely negative to other IF proteins.

Endothelial cells (EC) were vimentin-positive in all placental vessels (Fig. 4, 6 - *a, b*). The expression pattern of IF proteins in vascular myocytes (SMC) in different

placental vessels looked complicated. Vimentin has been expressed practically by all myocytes of larger vessels of the chorionic plate (Fig. 4) and villous trunk (Fig. 6 — *a*) but only in single SMC of smaller vessels (Fig. 6 — *a, b*). In principle, all vascular SMC reacted to desmin. The reaction intensity in the media of larger vessels of the chorionic plate and villous trunk increased in the direction to the periphery of the wall (Fig. 5 — *a, b*). It seemed even that most SMCs of the inner media expressed desmin very weakly. In the microcirculatory region all SMC of closed and/or partially muscular media (i. e. arterioles, pre-, postcapillaries, and venules) were desmin-positive (Fig. 5 — *c*). We did not observe cytokeratin immunopositivity in SMC.

Most villous stromal cells located closely to and independent of the vessel wall reacted positively to vimentin and desmin (Fig. 5 — *a, c*; Fig. 6 — *a, b*). They did not display cytokeratin positivity. This expression pattern was typical not only of the reticular stroma of small-calibre villi but also, to a smaller extent, of the fibrous stroma of the larger villi. In the latter all cells of the villous interior were vimentin-positive and most of those close to the vascular media or connected to medial SMC were intensively stained for desmin (Fig. 5 — *a*). We observed vimentin and desmin positivity in all stromal cells of intermediate and terminal villi (Fig. 6 — *b*, Fig. 7). The larger cells of the villous interior located more peripherally (presenting Langhans' rather than Hofbauer's cells) showed a weaker immunopositivity to vimentin and desmin but a strong staining for cytokeratin (vide supra). The distribution of IF proteins based on our findings has been systematized and presented in Table 2.

Discussion

Our investigation demonstrates that the cells of the human full term placenta synthetize IF proteins of different chemical nature. The distribution pattern of IF protein expression is of interest, especially regarding cell differentiation and function.

We observed cytokeratin-containing IF in all types of trophoblasts, i. e. in the cytotrophoblastic cells of different localizations, as well as in the syncytiotrophoblast. Cytokeratins are the first IF proteins that appear during prenatal ontogenesis [5, 19, 40, 42]. There were no differences between our own results and those of other investigations [2, 3, 20]. Thus we agree with the concept of Daya and Sabet [9] that cytokeratins can be considered as very sensitive and reliable markers for the trophoblast and its derivatives. Therefore we discuss the possibility for testing cytokeratin antibodies on the trophoblast. Cytotrophoblastic cells of the chorionic plate (Langhans' cells behave differently) express all types of IF proteins. They seem to retain their plural potency till the end of pregnancy. The syncytiotrophoblast synthetizes only cytokeratins [3, 20, 24, 30]. In our opinion, this characteristic is due to the early and complex differentiation of a syncytial borderline structure.

Along with the trophoblast cytokeratins were expressed by amnionic epithelial cells. Similar findings have been published by Regauer et al. [43], Khong et al., [20] and Beham et al. [2]. Concerning the amnion two points should be made: 1) the immunopositivity was not strong; 2) not all cells were stained. It is known that amniocytes contrary to other epithelial cells [see the table by Moll et al. [31], are heterogeneous concerning their cytokeratin composition [2]. It becomes clear that at least certain amniocytes, namely the PAN-cytokeratin-positive cells possess the genes encoding most of the cytokeratins. The presence of cytokeratins and their intracellular distribution (laterobasal) corresponds more to the secondary mechanical tasks of these cells in the epithelial entity. Evidently cytokeratin-containing cells serve main-

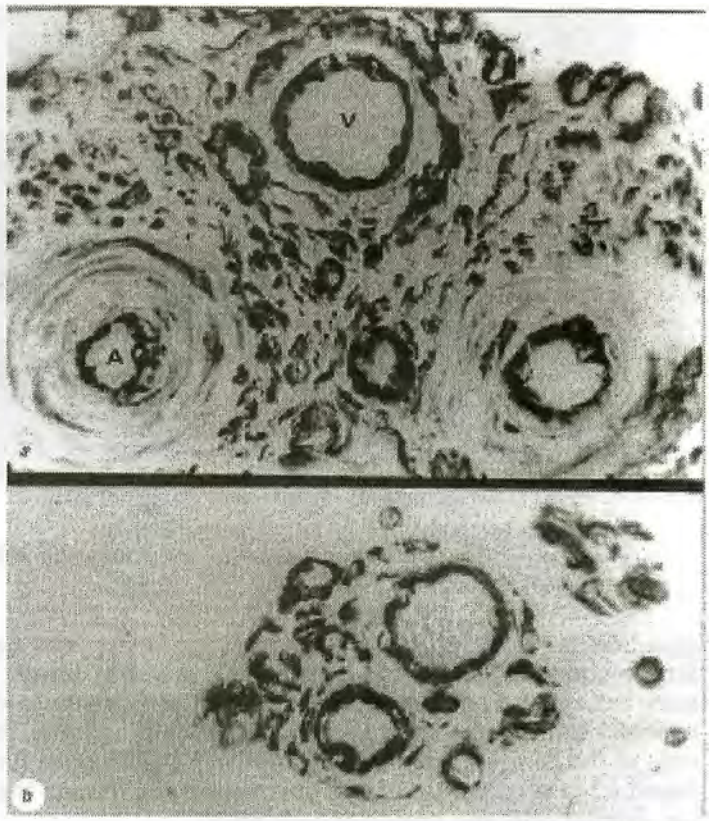

Fig. 6. Vimentin expression in a branch of a stromal villus (*a*) and of an intermediate villus (*b*)
A — small-sized artery; V — small-size vein (×400)

T a b l e 2. Distribution of IF proteins in placental cells

Placental cells (Localization)	Cytokeratin	Vimentin	Desmin	GFAP
Amniotic epithelial cells	+	-	+	-
Cytotrophoblast of the chorionic plate	+++	+++	+++	++
Syncytiotrophoblast	+++	-	-	-
Cytotrophoblast of the villi	++	+	+	-
Endothelial cells	-	+++	-	-
Myocytes of large-sized vessels	-	++	+++	-
Myocytes of small-sized vessels	-	+	+++	-
Villous stromal cells	-	+++	+++	-

ly for the integrity, stability and protection of the amnionic epithelium as a whole. We failed to identify any other localizations of cytokeratin as Khong et al. [20] — in vascular and villous stromal cells and Lifschitz-Marceil et al. [26] - cytokeratin 18 in pathologically altered placental vessels. It seems that cytokeratin-containing stromal cells described by Khong et al. [20] are free cytotrophoblastic cells.

Initially, vimentin has been considered characteristic for mesenchymal and connective-tissue differentiation marker [13, 40]. Other IF proteins have also been detected [49]. It is the endothelium that expressed vimentin as the only cellular IF protein. Such an expression is characteristic for other vessel endothelial cells of the hu-

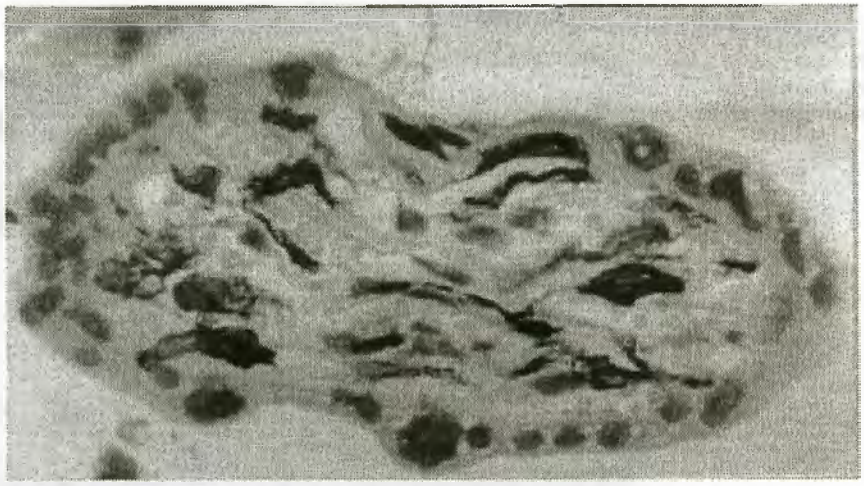

Fig. 7. Desmin-positive stromal cells in a terminal villus
The cytotrophoblast shows desmin positivity, too. Poststaining with Mayer's haematoxillin (×400)

man body as well [13]. Vimentin immunopositivity was expressed also by SMC of large blood vessels [see also 12, 39, 40, 45], as well as desmin [see also 5, 21, 24]. Contrary to Cremer et al. [8], Khong et al. [20] and Beham et al. [2] we did not observe vimentin-immunoreactivity in amniocytes while, as a rule, the free cytotrophoblastic cells of the chorionic plate have intensively been stained for vimentin (Table 2). We found a repeated co-expression of vimentin and desmin that is first of all typical of the stromal cells and myocytes (vide infra).

Desmin was expressed (more weakly) only by single amniocytes. Free cytotrophoblastic cells of the chorionic plate reacted much more intensively to desmin. Besides, these cells were the only placental cells in which for the first time we were able to show GFAP-immunopositivity. The SMC of all vessels and the villous stromal cells showed consistent immunopositivity to desmin. It is an interesting feature that endothelium-close SMC reacted weakly compared to those of the outer media. Nanaev et al. [34] consider desmin as a reliable marker for muscle differentiation while Sparn et al. [46] revealed desmin-positive stromal cells which were negative for a-smooth muscle cell actin. It is possible, that outer-media SMC and most stromal cells undergo a similar myocytary differentiation. Data on the synthesis of alpha-smooth muscle cell actin and myosin by most stromal cells and our own unpublished data (along with desmin and vimentin) may argue in support of this statement [2, 21, 22, 26, 35]— Kohnen et al. [21] assume that such cells of the trunk villi differentiate contractively from vascular media SMC. We agree with this concept. An argument in support of this may also be the strong desmin immunoreactivity of outer media SMC which remain more myocytically differentiated till the term of pregnancy, compared to those of the inner media. The myocytic differentation in the villous tree is directed from the stroma towards the vascular lumen. This statement is, however, valid only for levels higher that the microcirculatory system of the placenta. All their extraendothelial vascular-wall cells were desmin-positive. They are believed to be true contraction/relaxation-capable myocytes. Preceding from our and other data

available [11, 21, 22] we can suppose that desmin-positive vascular-wall myocytes as well as stromal cells [i.e."myofibroblasts" in the sense of Gabbiani et al. [15], represent a united autonomous contraction/relaxation system of the villi capable to adapt motor function of the small-size villi. As the placenta lacks nervous supply [4, 36] this system has to react to different mechanical and chemical stimuli such as local release of EDRF [6, 16, 32, 33, 37] and other substances [28, 48]. It most probably maintains a dilatatory tone and relates microcirculation to function.

Acknowledgements: The authors wish to thank Mrs M. Boshkova, Sn. Bachvarova, Sn. Vladimirova, P. Stankova and Zl. Dimitrova for their skilful technical assistance and Dr. D. Tomov for the manuscript preparation.

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