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IMMUNOHISTOCHEMISTRY STUDY ON CITOARCHITECTURAL MODIFICATIONS AT PLACENTAL LEVEL IN CASE OF DEVELOPMENTAL DISORDERS AND PREMATURITY

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The hereby study aims to be a retrospective, a summary of a study on placental structural changes outlined by new researches from more than ten years. The studied lot has a statistical value being balanced, with the clinical laboratory and evolutionary arguments sufficient to allow an accurate analysis, and is representative of the studied problems, represented by morphological changes that correlate with different physiological placental variety of impaired intrauterine growth and development and perinatal consequences. Also the lot allowed through the included categories to make an analysis of placental citoarchitecture and of the fetoplacentar unit in order validate the study findings. I made these remarks because in multiple situations the analysis of morphological placenta was insufficient or superficial, losing the valuable information on product evolution and prognosis from a conceptual task with increased risk or no risk. Immunohistochemical study shows superiority in identifying structural changes of cellular and tissue intimacy at the placental level compared with morphological and histochemical methods.

Key words: immunohistochemistry, monoclonal antibody, placental citoarchitectonics, perinatal pathology.

Assumptions study was selected from the clinic of obstetrics and neonatology "Salvator Vuia" of the Medicine Faculty , University "Vasile Goldis" Arad. Selection of cases was to mark the main objective of the study, namely the existence of major disturbances of intrauterine growth, both general (prematurity / intrauterine growth delay) and local or systemic objectified by the existence of a pathological malformation. Trough this were tempted to find and establish correlations between morphological changes and placental changes (mostly microscopic) and some relevant parameters of fetal and perinatal period with potential impairment of fetus and possible generating growth retardation of the intrauterine development reflected in a subsidiary placental pathology. The selection of cases for the study group and control group was necessary in order to realize a controlled statistical consideration which is important to further study the course of the morphoclinical placental changes and provide them with the central objective of the research. Our casuistic has been placed in the general context of the increasing incidence of malformation pathology associated with intrauterine growth retardation and / or prematurity, in other words malformative pathology,which generally interfere significantly with the growth and fetal development. Today many European countries statistics indicate a percentage of 11.2% of malformative syndrome and almost a double incidence of prematurity from different causes. After Altschuller "placenta is similar to a log of gestational life". Conventional journals often contain non-important information, but others contain obscure information that are ignored and are responsible for adverse results to come." Thus our attention was focused on studying the placenta convinced that "the placental universe" it's condensed and complex and still hide unknown information, which after will be resolved and put in correlation with malformative pathology and prematurity will lead to their decrease through a medical operative attitude. Based on these assumptions placental factor research is fully justified on the one hand due to the fact that the pathogenetic chain links in the situations described are far from being clarified and on the other placenta seen in the modern sense is not simply a transient embryonic Annex but an organ generated by pregnancy with a very important role.

METHODS AND MATERIAL

Were harvested immediately after the macroscopic analysis and placental atropometrics, placenta blocks about 1 / 1 cm both on the fetal and maternal placental faces. Histological cups were made of pieces collected by conventional methods with inclusion in paraffin and blocks realization, after which were made sections and mounted on specific slides.

The pivotal segment common to all immuno-histochemical techniques is the antibodies. The availability of the immunoglobulin fraction and monoclonal antibodies in an increased context of tissue antigens increases greatly the quality and quantity of imunohistologic repertoire. We believe that in order to better understand potential immunohistochemical staining methods, the reasons why we chose them as any latent problems that may be associated, we are making the summary of basic knowledge of antibodies and their potential, their scientifically acceptable limits, indicated by DAKKO international system. Monoclonal antibodies

are the product of an individual clone of plasma cells. Antibodies derived from a given clone are identical immunochemical and react with an epitope specific for the antigen for which are formed. In immunohistochemistry, there are some advantages that monoclonal antibodies have on the polyclonal ones.

These include high homogeneity, absence of non-specific antibodies, facilitate the characterization does not present too high variability.

Monoclonal antibodies are planned for the formalin fixed specimens. For this reason, target epitope must survive fixation the.

The epitope in question, must be unique to a particular antigen. Specificity, one of the greatest benefits of monoclonal antibodies, is lost if the antibody is directed to an epitope shared between two or more antigens.

The antibodies derived from hiperimmunizied animals not only differ in terms of what determinants recognize multivalent antigens, but differ in their affinity for the same. The word "affinity" was used to describe both the functional and intrinsic affinity.

Intrinsic affinity of an antibody is in the HV region and is determined by its amino acid sequence that confers specificity.

One can say that with greater specificity, affinity is strongest, without unduly simplifing, because ionic interactions, hydrogen bonds and van der Waals forces are the major factors contributing to the intrinsic affinity between the antibody's paratop and the antigen's epitope.

As the amount (titer) of antibody increases over time during immunization, the quality (affinity)increases also. This process iscalled "affinity maturation". Low immunogenic doses will increase the rate of maturation of affinity, but will reduce antibody levels and vice versa.

In immunohistochemistry, functional antibody affinity is defined by the amount of time passed until the equilibration with the tissue antigen. The term "greed" was used synonymously to describe the functional affinity, but also to show the strong link between antibody and antigen. Because antigen antibody reactions are reversible, simple immune complexes formed on the tissue could be separate in washing cycles used in immunohistochemistry. The ease and degree to which this dissociation occurs, vary from antibody to antibody and low salt concentration and low temperature, will reduce the likelihood of the weak staining due to the dissociation of an already formed immune complex.

High affinity antibodies are preferred and have the advantage that during washing, dissociation is less likely to occur.

On the other hand, monoclonal antibodies have an affinity even if the affinity is low and loss of coloration is probably due to its dissociation of epitope from antibodies. As stated, we attempted to avoid factors that weaken the link between antigen and antibody such as high salt concentrations, high temperature and low pH during washing of the specimens. We relied on the experience of our employees in handling antibodies, whom know that washing and incubation can be reduced to safety margins and a slight shaking helps to reduce background staining. Monoclonal antibodies, whether of high or low affinity, can not form a consistent environment with antigen and, rarely form insoluble precipitates. However, in immunohistochemistry, the ability antibodies to form precipitate immune complexes is of minor importance, because tissue reaction with immobilized antigen, presents the antibody capture by the tissue, rather than precipitate. In immunohistochemistry the optimal antibody titers can be defined as the highest dilution of antiserum (or monoclonal antibody), which will result in a specific color with the lowest maximum amount of background under a specific test.

The highest dilution is primarily determined by the absolute amount of specific antibodies.

For preparations of monoclonal antibodies, the absolute concentration of antibodies can be measured by reading, and often these form the basis to reach the required dilutions.

The highest dilution is also governed by the intrinsic antibody affinity; if the titer is maintained constant, a high affinity antibodies will probably react more quickly to tissue antigen and give a more intense staining in the same period of incubation, then an antibody with low affinity.

Proper dilutions contributed to the quality of staining which are accurately prepared.

Were determined by first selecting a fixed incubation time, and then making small amounts of experimental dilution series.

Depending on the size of the specimen application from 0.1 to 0.4 ml of the section, were generally adequate.

On paraffin sections, optimal dilutions of primary antibodies were not only marked by the peak intensity of coloration, but by the presence of minimal background. Once established optimal dilution were prepared in larger volumes based on needs and stability.

The limit to which monoclonal antibodies can be diluted, is the subject of additional criteria, which we do not address it here.

Because of the restricted molecular conformation, monoclonal antibodies are more sensitive to pH and solvent ions.

It was therefore proposed that any evaluation of monoclonal antibodies include their titration at pH 6.0 and 8.6 in the absence of NaCl.

This high dilution with high pH,avoiding the highest immunoreactivity was named optimal dilution and was recommended for use. Immunoenzymatics staining methods uses enzyme-substrate reactions to convert colorless chromogens into a finished colored products.

Among the enzymes used in these applications, we chose peroxidase. Selecting a suitable enzyme for a

specific immunohistochemical application depends on a number of criteria:

- Enzyme must be in a highly purified form and has to be cheap
- Conjugation or noncovalent links must does not lead to abolition of enzymatic activity, although it might diminish it.
- Enzyme needs to be stable in solution
- Endogenous enzyme-activity to intersect only minimal with antigen-specific stains.
- Enzyme-reaction products to be easily detected and determined.

Radish peroxidase and alkaline phosphatase are meeting most of these conditions. Radish peroxidase in the presence of an electron donor, will form first enzyme-substrate complex and then oxidation of electron donors. Electron donor is to promoter of the catalyzation of H2O2, while its absence effectively stops the reaction. There are several electron donors which after oxidation become colored products, and are named chromogens.

This property and the insolubility after oxidation, make this donors useful in immunohistochemistry electro. We used the 3,3'-DIAMINOBENZIDINA (DAB) system.

This leads to a brown finished product that is strong insoluble in alcohol and other organic solvents. DAB oxidation and polymerization thereby causing the ability to react with the osmium tetroxide and thereby increasing the intensity of staining and electron density, however. A key part of cytological and histological techniques is the preservation of tissues and cells in a manner close to situ conditions.

To achieve this objective, blocks of tissue, large sections spread on slides were immersed in liquid fixative (provided in DAKKO kit), although in the case of large, only the dry of the preparation acted as a form of preservation.

The fixing substance, prevent autolysis by inactivating lysosomal enzymes and inhibits the growth of bacterial cultures which could pose risk of changes in decay. Moreover, the fixing substance, stabilizes cells and tissues to protect them from the rigors of subsequent processing of the staining technique.

I could not choose for other setting, because in laboratories where I worked was only the formaldehyde on the bed of paraffin. LSAB preparations are applied in sequence of the primary antibody of rabbit (mouse),anti-rabbit immunoglobulin (anti-mouse) and conjugate streptavidina enzyme.

Color reaction is then obtained with suitable substrate.

The working methods used is DAKKO LSAB 2 System. This system is for rabbit or mouse monoclonal antibodies,used in qualitative identification of antigens through simple microscopy and IHC on paraffinated tissues , and cryostat tissue and cell preparations in order to facilitate the diagnosis of pathological situations (paraffinated tissues) . IHC staining techniques allow visualisation of the antigens from the tissues.

These techniques are based antibodies immunoreactivity, and chemical properties of enzymes or enzyme complexes, which reacts with chromogens colorless substrate to produce a final colored product. IHC stain sensitivity was significantly improved with the advent of indirect techniques. In this two-step method, secondary antigens linked with enzyme, reacted with primary antibody bound to antigen.

Further, we managed an increase in sensitivity by introducing antiperoxidaza-peroxidase (PAP)enzymatic complex. In this method the secondary antibody serves as a link antibody between primary antibody and PAP complex. Subsecvențiale developments in IHC exploited avidin strong affinity for biotin and as a result we obtain avidin-biotin complex(ABC).

This technique using avidin-biotin enzyme complex, which is mixed before use to form another complex with biotinated secondary antibody . This method improved the sensitivity of the preparation compared with PAP method.

HRP DAKO LSAB2 is based on avidin-biotin labeling tehnique,(LAB), in which a biotinated secondary antibody forms a complex with the molecules of streptavidine conjugated with peroxidase. Compared with the ABC method, is up to4-8 times more sensitive. DAKO LSAB HRP ,is an IHC sensitive procedure that allows simultaneous access of several specimens with rabbit or mouse primary antibodies,in less than one hour.

Endogenous peroxidase activity is inhibited by incubating the specimen for 5 minutes in 3% hydrogen peroxide.

The specimen is incubated with a rabbit or mouse primary antibody diluted and characterized, followed by sequential incubation for 10 minutes with biotinated antibody and streptavidină labeled with peroxidase.

The staining is successful after incubation with substrate chromogen (AEC or DAB, we used DAB).

In the case of DAB we talking about an incubation for 5-10 minutes with 3-3' diaminobenzidine, resulting in a brown precipitate at the place of the antigen. For a better adhesion in the IHC staining procedures were used only microscope slides coated in poly-L-lysine, because they are unique recommended at proteolytic digestion procedures and disclosure target. IHC is a diagnosis process that requires specialized training in the selection of suitable preparations;tissue selection, fixation and processing; preparation of the IHC slide; interpretation of staining results. Tissue staining is dependent on proper handling and processing of tissues prior to staining. Incorrect fixing, freezing, washing, drying, heating, sectioning or contamination with other tissues or fluids may produce artifacts, antibody masking or false negative results. Inconsistent results may be due to changes in methods of fixing or irregularities inherent in the tissue. Clinical interpretation of any positive stains

or its absence must be complemented by morphological and histological studies with proper controls.

It is the responsibility of a qualified pathologist to interpret the color preparations. Staining was done in collaboration with the University licensed medical laboratory and pharmacy in Timisoara, which is responsible to review the slides and to ensure the extent to which positive and negative control is adequate.

In the present study we used six monoclonal antibodies that we will present at firm indication as a specification before we present the obtained results.

Results of immunohistochemical stain of placentas.

Immunohistochemical study of placental fragments showed metabolic activity of the trophoblast,at the sincitial level and at the citotrofoblastic levels of the chorionic villi. Through the immunohistochemical stain were identified the citotrophoblast of the columnar and basal cells, interstitial trophoblast that invades basal"decidua",trophoblast giant cells that migrate into the myometrium, endovascular citotrophoblast. These methods have allowed the identification of listed structures which are constantly reshaping and restructuring (constructive or destructive) the levels in placental tissues.

Citokeratine

Name: monoclonal mouse; human anticitokeratine, AE1-AE3 clones

Immunogene: human epidermal callus

Class / subclass: IgG 1 Kappa

Code: N1590

N-Series DAKO

Primary Antibodies

Use with DAKO Envision, DAKO Envision System LSAB2 Doublestain and DAKO. Designed for in vitro diagnosis.

Human anticitokeratine monoclonal antibody, AE1/AE3 clones, is intended for qualitative identification of two epitopes present on most normal tissues or in epithelial citokeratines neoplasms, using immunohistochemical testing methods. Citokeratines are a family of water-soluble proteins, with molecular weight between 40-70 kd,which form the cytoskeleton of epithelial cells. AE1/AE3 is a mixture of two monoclonal antibodies obtained by immunizing mice with human callus keratin. DAKO AE1/AE3 was able to identify most human citokeratines and can be used as a tool to identify cells of epithelial origin,simple or stratified. AE1 antibody reacts with an antigenic determinant present on most citokeratines of subfamily A, including citokeratines with Moll 10,13,14,15,16 and 19 destination, but not 12, 17 and 18. AE3 antibody reacts with an antigenic determinant found in citokeratines of subfamily B, including the number 1 and 2, 3, 4, 5, 6, 7 and 8. This primary antibody is obtained in volumes of 7 and 11 ml., as a tissue supernatant culture of mouse antihuman monoclonal antibody in 0.05 M Trsi-HCl, pH 7.6 containing 15 mM NaN3 and fetal bovine serum protein.

This product is intended to DAKO Envision,DAKO Envision Doublestain and DAKO LSAB.

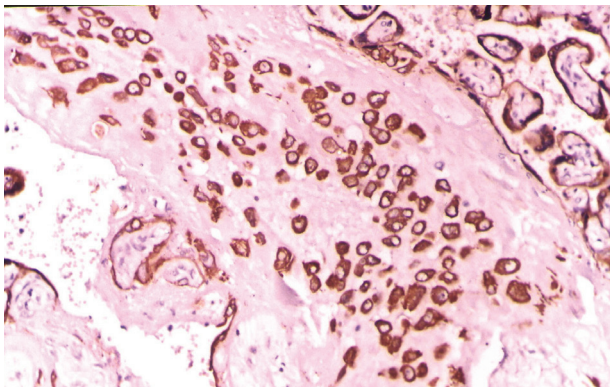

Figure 1. Positive reaction for CK in the citotrophoblast of the basal plate(X cell mass of fibrinoid necrosis) (LSAB antiCK X 200)

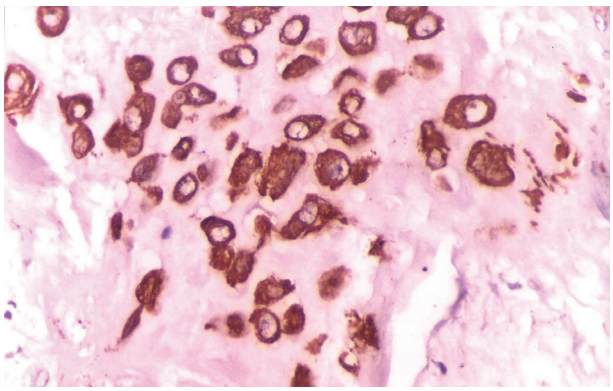

Figure 2. Detail from previous figure (LSAB antiCK X 400)

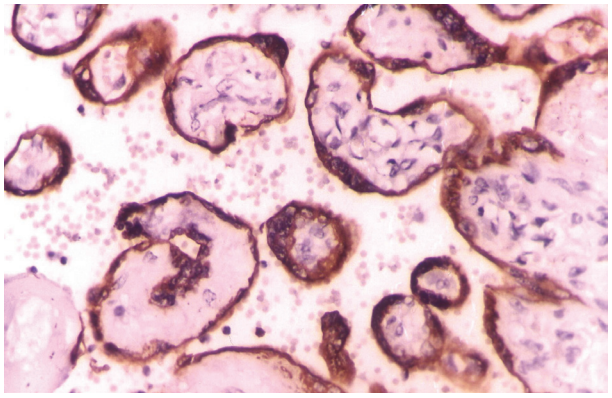

Figure 3. Trophoblast with positive reaction for CK: syncytial buds and nodules, intravillosital trophoblastic buds with positive reaction, villi with fibrosis axis, hyalinization (LSAB antiCK X 400)

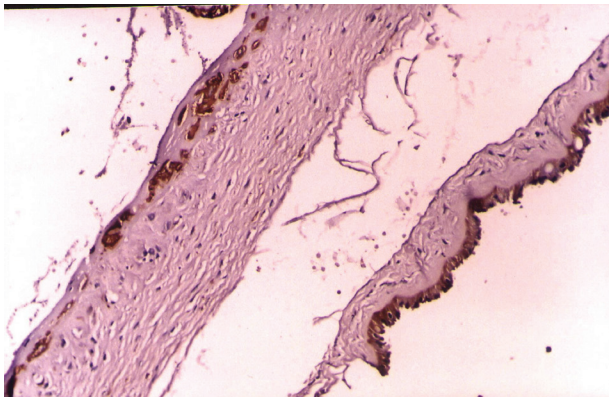

Figure 4. Chorionic plate, heterogeneous positive reaction for CK in the amniotic epithelium and chorionic citotrophoblast with no reactivity at the fibrous level, fibroblastic level,rare fusiforme cell reactive in the fibroblastic layer of the chorion. Amniotic epithelium with vacuolation and slightly disorganized. (LSAB antiCK X 200)

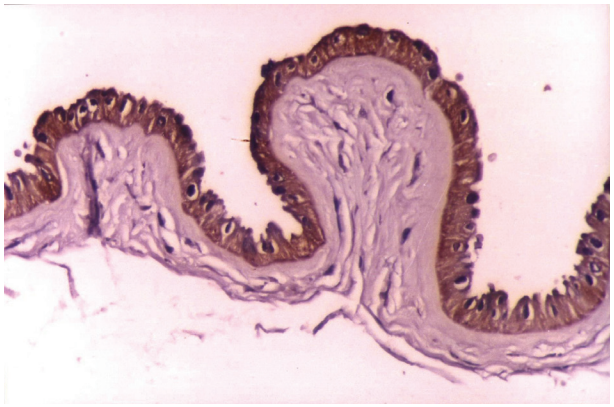

Figure 5. Positive reaction for CK slightly heterogeneous of the amniotic epithelium, the absence of reactivity in the connective tissue of „amniosului". (LSAB AntiCKX400)

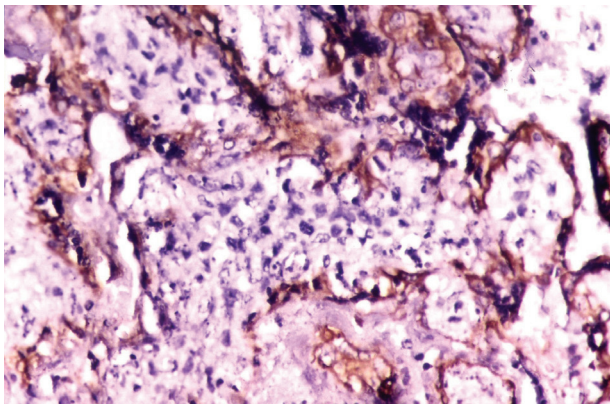

Figure 6. Positive reaction for CK of weaker intensity in the case of trophoblast villi. Inflammatory elements in the villozital axis, chorionic agglutinated. (AntiCK X 400)

Vimentine

Name: monoclonal mouse, anti-vimentine, VIM 3B4

Immunogenicity: purified vimentine bovine clone: Vim 3B4.

Class / subclass: IgG 2a, kappa

Code: N1583

DAKO N-Series

Primary Antibodies

use with DAKO Envision , and DAKO LSAB DAKO Envision Doublestain

Designed for diagnosis in vitro

This antibody is intended for the laboratory use to identify by simple microscopy mesenchymal cells in normal tissues and neoplasms using immunohistochemical testing methods. Positive results helps in classifying tissue neoplasms, for example melanoma. Differentiated identification is facilitated by the results of the panel of antibodies.

Normal tissues: Azumi and Battifora showed the distribution of vimentine's in most normal mesenchymal cells as fibroblasts, smooth muscle cells, adipocytes, Schwann cells, vascular endothelial cells, macrophages, myoepithelial cells as the salivary glands, sweat glands and breast glands.

Cellular vimentine distribution and intensity of coloration, were more variable in thyroid follicular epithelium, adrenal cortex, renal distal tubule epithelium and mesangial and endothelial cells of glomeruli, and also in pancreatic acinar cells. Also some large lymphocytes showed cytoplasm immunoreactivity.

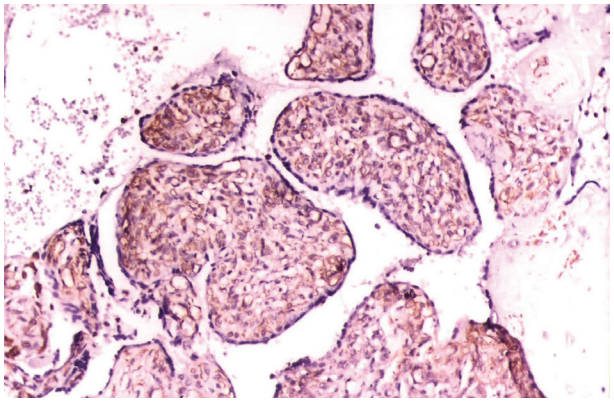

Figure 7. Villi with cellular stroma rich with positive response to vimentine of the stromal and endothelial cells of immature placenta. (LSAB anti-vim X 200)

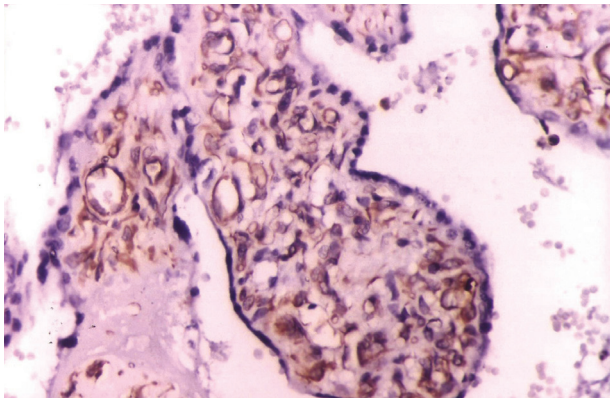

Figure 8. Detail of the previous picture (LSAB anti-vim X 400)

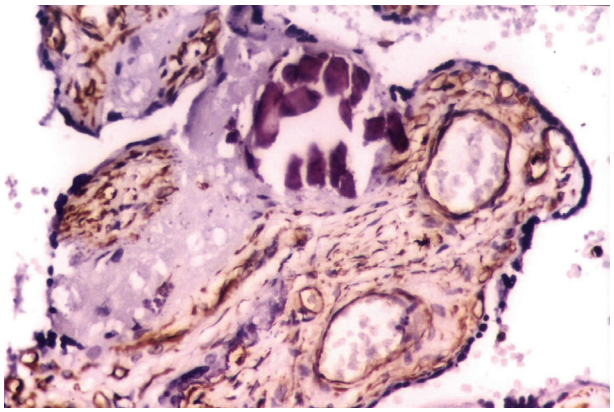

Figure 9 .Stromal and endothelial cells with positive reaction for VIM, absence of reactivity at the trophoblastic level and the outbreak of fibrinoid necrosis,calcification. (LSAB anti-vim X 400)

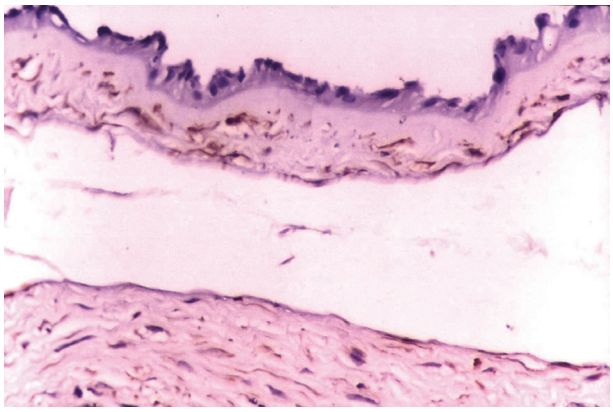

Figure 10. The absence of reactivity in amniotic epithelium, positive reaction in the conjunctival subcorial cells. (LSAB anti-vim X 400)

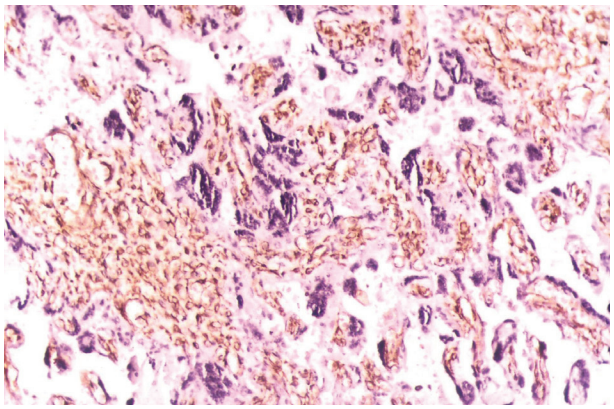

Figure 11. Agglutinated villi, symphyse with syncytial sprouts, positive reaction for VIM in blood vessels and stromal cells increased in number. The absence of trophoblastic reactivity (LSAB anti-vim X 200)

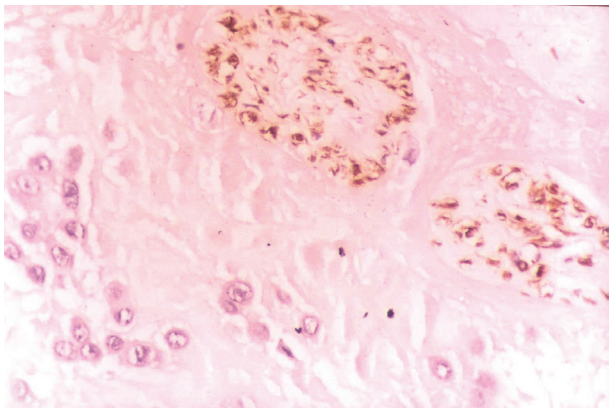

Figure 12. Positive reaction for VIM in the villi spindle cells, constrained by fibrinoid material, X cells with no reactivity (LSAB anti-vim X 400)

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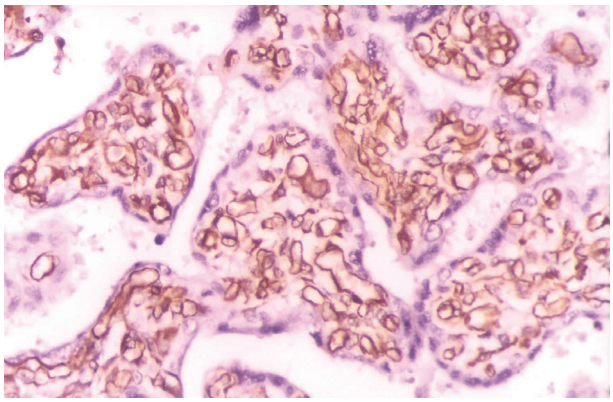

Figure 13. Villi with corangiosis aspect (anti-vim x 400)

CD-34

Name:monoclonal mouse , human hematopoietic progenitor anticell, QBEnd 10 clone

immunogene: human endothelial venules Class / subclass: IgG 1 Kappa

Code: N1632-CD34

DAKO Cytomation N-Series

Primary Antibodies

Use with DAKO EnVision, DAKO Envision Doublestain and DAKO LSAB

Code: NP036 NP-Series DAKO Cytomation Primary Antibodies

DAKO EnVision +DAKO LSAB + Systems Designed for diagnosis in vitro.

This antibody is used for qualitative identification by simple microscopy of hematopoietic progenitor cells and endothelial cells. The antibody is valuable in the identification and characterization of vascular tumors leukemia. Differentiated identification is facilitated by the results of the panel of antibodies.

Normal tissues: The antibody will react with interstitial dendritic cells present in many organs, vascular endothelial cells, 1% of mononuclear bone marrow cells and rarely with myometrium. Anti CD-34 labeled vascular endothelial cells in papillary dermis of normal human epidermis. Endothelial cells of blood vessels and post capillaries venules and capillaries arterioles are also colored. Basement membrane region of the human gland lymphoid cells and hematopoietic progenitor cells are also labeled.

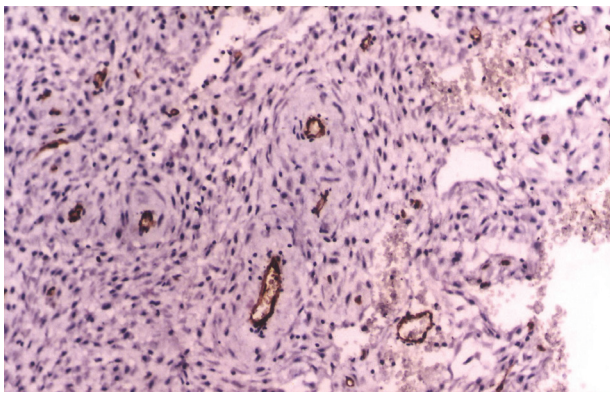

Figure 14. Vascular endothelium positive for CD 34. (LSAB anti CD34 X 400)

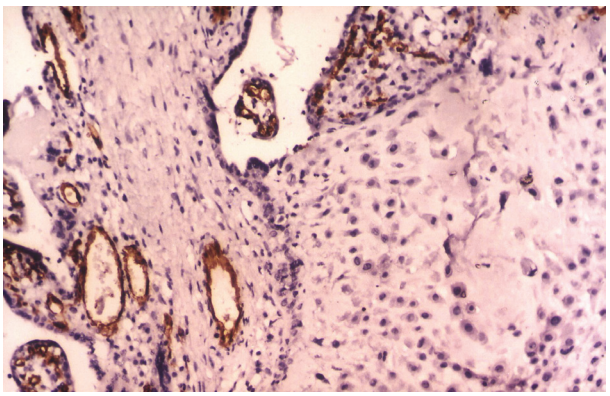

Figure 15. Positive reaction for CD 34 in vascular endothelium, the absence of reactivity in the epithelium and trophoblastic cells X (anti CD34 LSAB X 200)

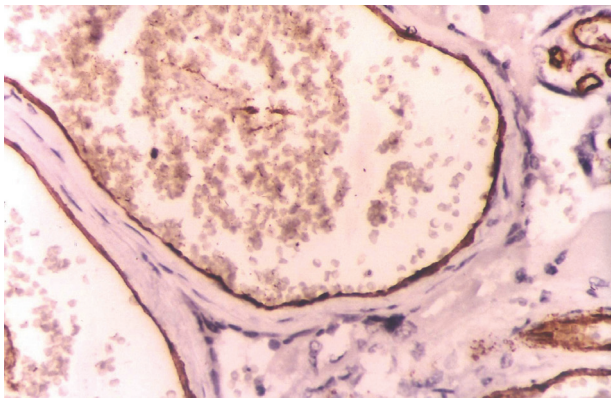

Figure 16. Positive reaction for CD 34 in vascular endothelial placenta (anti CD34 LSAB X 400)

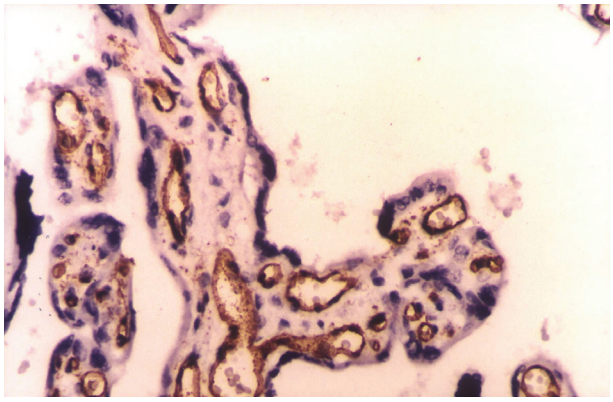

Figure 17. Villi enclosed by a sinctio-plasmatic frame, villi vessels with positive reaction for CD 34 (LSAB anti CD34 x 400)

Actin

Name: monoclonal mouse, human muscle anti-actin,HHF35 clone

Immunogenicity: SDS protein fraction of human myocardium

Class / subclass: IgG 1 Kappa

Code: N1567

DAKO N-Series

Primary Antibodies

Use with DAKO EnVision, DAKO EnVision doublestain and DAKO LSAB

Designed for diagnosis in vitro.

This antibody is intended for the qualitative identification of a muscle actin epitope present on normal and malignant tissue using immunohistochemical testing methods. HHF35 clone was shown to be an effective marker for soft tissue tumors with muscle differentiation such as leiomyoma, leiomyosarcoma and rhabdomyosarcoma. Differentiated identification is facilitated by the results of the panel of antibodies.

Actin, a cytoskeleton protein muscle cells and nonmusculare, exists in three amino acid sequences that differ by izotypes and their isoelectric points. Anti-actin monoclonal antibody human clone was obtained by immunizing mice HHF35 a polypeptide fraction of human myocardium suffering from hypertrophic stenosis idiopathic sub-aortic.

HHF35 anti-actin antibody, does not react with alpha-actin in non-muscle sources. It has been proven the specificity of HHF35 for alpha and gamma izotypes of skeletal muscle, cardiac muscle and smooth muscle.

HHF35 in normal tissue, stains at cytoplasmic level the skeletal muscle fibers, smooth muscles of arteries, veins ,muscular tunic of the gastrointestinal tract, uterine myometrium, prostate stroma, capsule cell of several parenchymal organs, including liver, kidney, lymph nodes and spleen, and the myoepithelial layers of the ducts and mammary glands. Other non-muscle cells are non-reactive, including vascular endothelial cells, epithelial cells, lymphoid cells, macrophages, connective tissue and nerve cells.

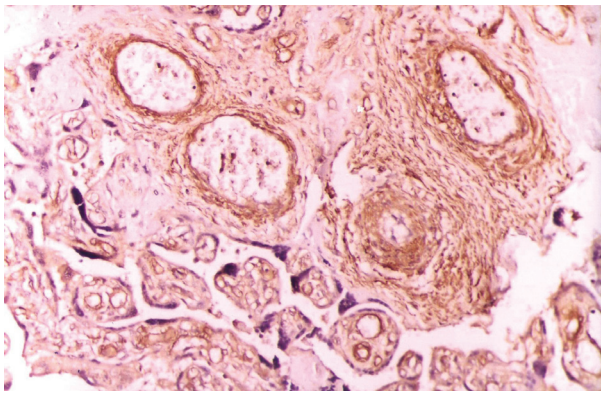

Figure 18. Positive reaction for the actin from the wall vessels of villosital trunks and perivascular cells (LSAB anti-actina X 200)

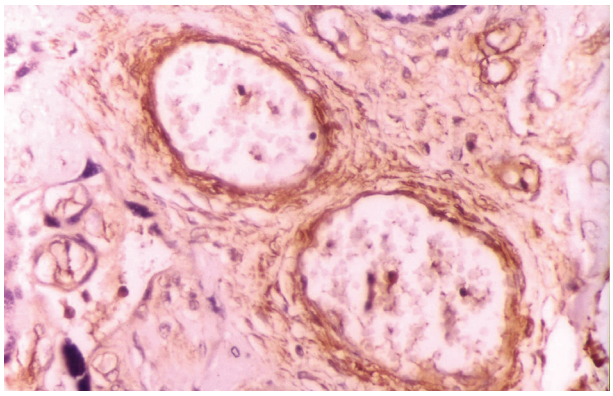

Figure 19. Detail of previous picture (LSAB anti-actina X 400)

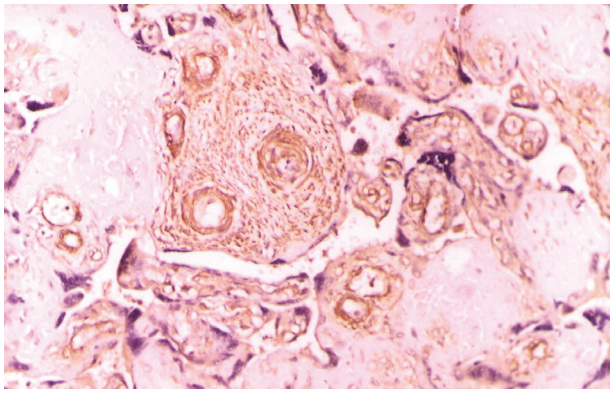

Figure 20. Increased number of reactive cells for actin in the vessel wall and perivascular chorionic trunks. (LSAB anti-actina X 200)

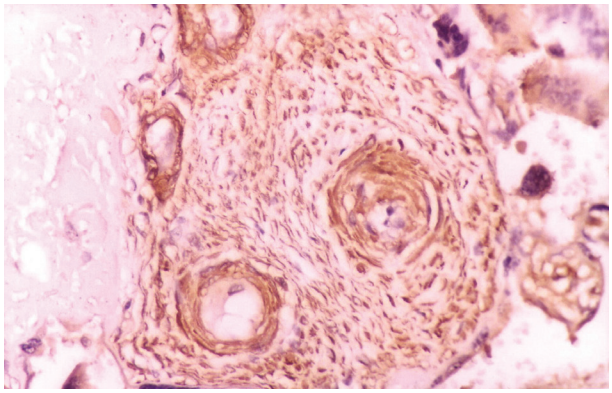

Figure 21. Acti VI 17 Increased reactive cells for actin in the vessel wall and perivascular villosital trunks. (LSAB anti-actina X 400)

KI - 67

Name:monoclonal mouse,human anti Ki-67 Immunogenicity: recombinant

peptide corresponding to 1002 bp Ki-67cDNA

Clone MIB-1

Class / subclass: IgG 1 Kappa

Code: N1633

DAKO N-Series

Use with DAKO EnVision NP-Series , DAKO EnVision Doublestain+DAKO LSAB

Code: NP011

Use with DAKO NP-Series, DAKO EnVision +DAKO LSAB +

DAKO NP-Series, DAKO EnVision + si DAKO LSAB +

Primary Antibodies

DAKO EnVision + and DAKO LSAB + Systems

Designed for diagnosis in vitro.

Ki-67 antibody is used in the laboratory for qualitative identification of Ki-67 positive cells in normal or cancer tissue using immunohistochemical testing methods. Positive results indicate the passage of cells through cell division and normal tissue neoplasm. Differentiated identification is facilitated by the results of the panel of antibodies.

MIB-1 monoclonal antibody reacts with Ki-67 antigen associated with cell proliferation during the cell cycle and is absent in cells in G 0 phase. Antibody recognizes native Ki-67 antigen and recombinant segments of the molecule without Ki-67.

This product is intended DAKO Envision, DAKO LSAB and DAKO Envision Doublestain. Cellular staining pattern of antibody is nuclear. Normal tissues: mesothelial cells 3 / 3.

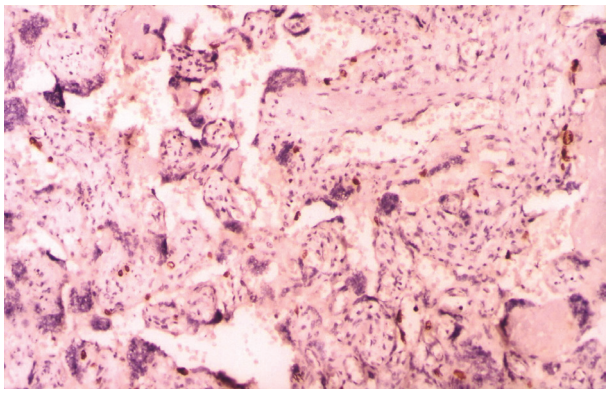

Figure 22. Agglutinated villi, symphysised, with fibrinoid stroma, with frequenct syncytial buds and moderate number of positive Ki-67 nuclei in the citotrophoblastic cells (LSAB Anti Ki-67 X 200)

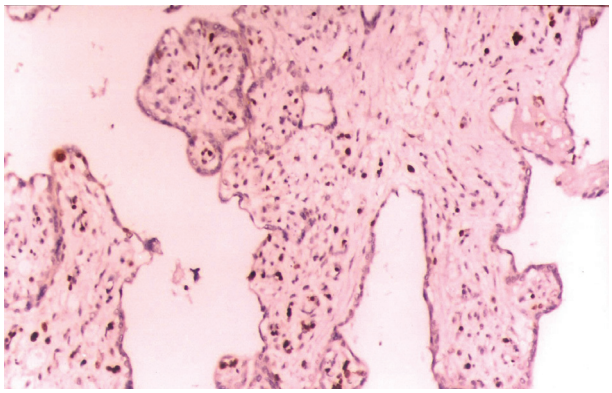

Figure 23. Numerous Ki-67 nucleus positive in trphoblastic cells and in chorionic stromae of the imature villi from a term placenta (LSAB Anti Ki-67 X 200)

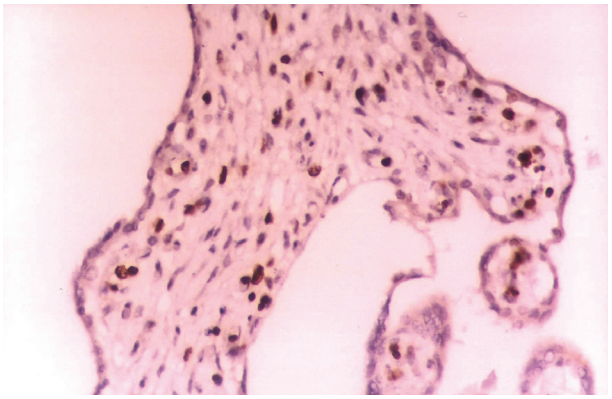

Figure 24. Positive reaction in the villosital stroma cells (detail) (LSAB Anti Ki-67 x 400)

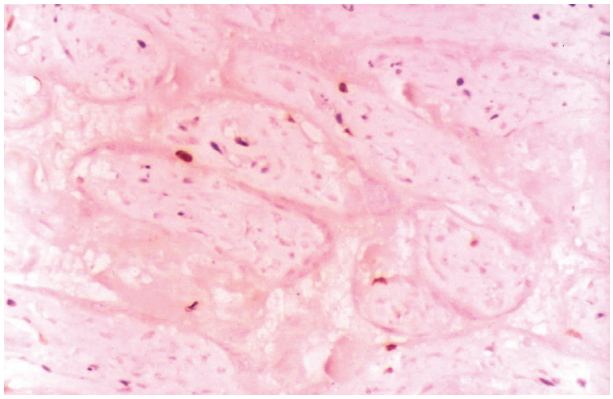

Figure 25. Necrosis of the villi with few nuclei positive nuclei for Ki-67 on the periphery of the villi (LSAB Anti Ki-67 x 200)

AFP

Name: Polyclonal rabbit,human anti-alfa1fetoprotein. (AFP) AFP Immunogenic human AFP.

Code: N1501.

DAKO N-SERIES

Primary Antibodies

For use with DAKO Envision, DAKO Envision Doublestain and DAKO LSAB2Systems. Intended for diagnosis in vitro.

This antibody is useful for qualitative microscopy to identify single cells containing AFP in fetal and malignant tissue using immunohistochemical testing methods. Positive results would help the classification of carcinomas. AFP is a glycoprotein containing 590 amino acids and has a molecular weight of 70 kd. It is constructed of a single polypeptide chain with three repetitive domains. AFP exon of the gene domains are very similar to the albumin. This product has been optimized for use in LSAB2 DAKO, DAKO Envision, DAKO Doublestaine Detection Systems or Dako Doublestain Detection Systems.

The cellular coloring model of AFP anti-human is the cell cytoplasmatic. Normal tissue: diffuse, granular cytoplasm stains with antihuman AFP can be seen in fetal liver cells and fetal intestinal tract. AFP is not present in normal non-fetal tissues. In normal tissue, human, adult, AFP expression is found not only in the fetus.

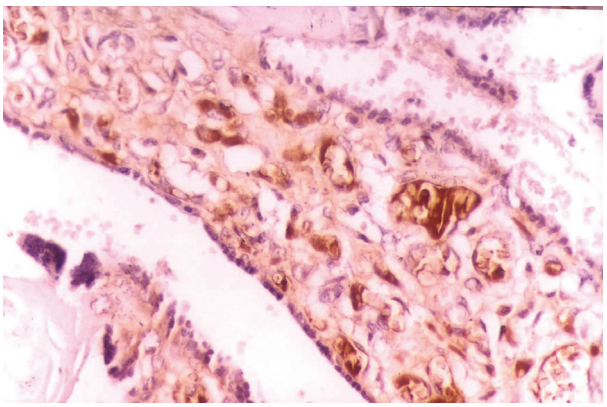

Figure 26. Positive reactivity for many areas of alpha-FP in the lumen of villosital vessels (alpha-FP LSAB x 400)

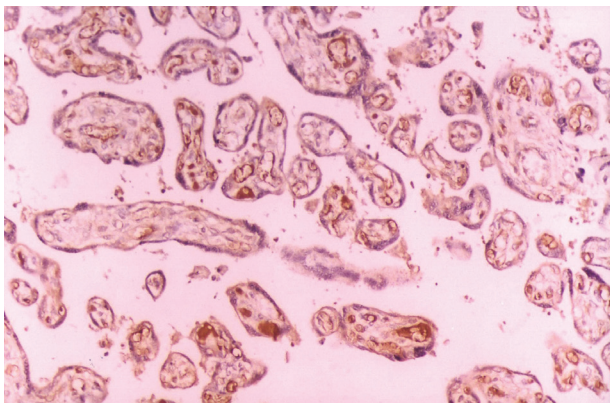

Figure 27. Small villi, mature, with frequent areas reactive for alpha-FP (alpha-FP LSAB x 200)

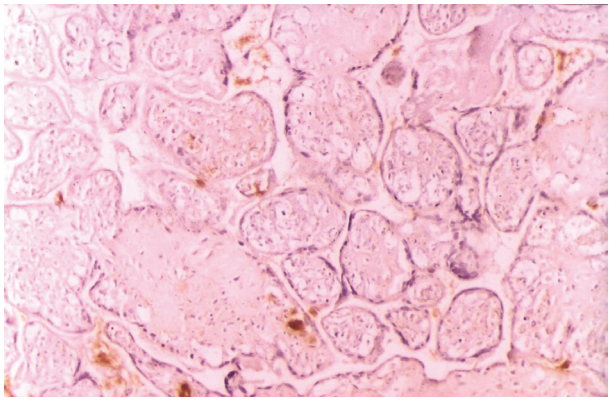

Figure 28. Villi with imflamatory fibrosis, alpha-FP focal reaction (alpha-FP LSAB x 200)

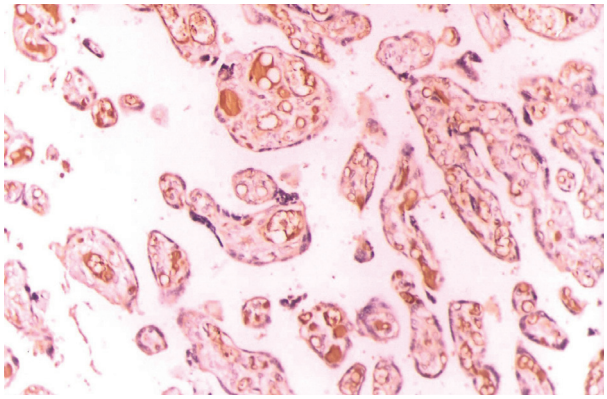

Figure 29. Small villi, mature, active in many areas for alpha-FP (alpha-FP LSAB x 200)

Immunohistochemical study of placental fragments revealed the following, depending on the antibody or cocktail of antibodies used:

CK - AE1/AE3 antibody marked as a final product of the reaction of amniotic epithelium brown cytoplasm with a uniform way of coloring, if normal amniotic epithelium and heterogeneous staining for amniotic epithelium suffering . I noticed at the fibroblastic layer of the chorion a few fuziforme fibroblast-like cells with cytoplasmic positive reaction for CK.(Fig 1,2,3,4,5,6)

On the cases analyzed, we observed an intense cytoplasmatic positive reaction for CK at the cyto-and syncytiotrophoblast level , without a large difference in reactivity depending on changes in villosity axis or depending on the nature of maturity or immaturity of the placenta .

We noticed also a strong positive reaction for CK in the X cells from fibrinoid necrosis mass .

Reactivity for CK is kept in areas of the placenta with ischemic changes, with the expansion of pseudoangiomatosis in chorionic vessels and in the case of necrotic villosity in placental area infarction, even after the disappearance of nuclei ; to be noted the weak positive reaction of the marker observed in areas of necrosis ..

In the case of placentas with changes of villitis and intervillitis we noted a weak reactivity for CK at the periphery of the villi, with focal sports restrained to a weak positive reaction, coupled with long-range destruction of trophoblast .

Vimentina: the marker was identified in all placentas in the study,in the following structures group placentas from fetuses / newborns with congenital malformation and control group: fusiform cells from the web , fibroblastic and spongious of "amnios" and from the placental villi , with no reactivity in amniotic epithelium. At the level of villi and villosital trunks we can see stromal cells and endothelial cells, with no reactivity in the trophoblast cells; the big vessels of the chorionic plate and of large stem villi presents a positive reaction for vimentin at the level of endothelial cells and to fusiform cells, arranged in concentric sleeves around vessels .(Fig.7,8,9,10,11,12,13)

X cells from fibrinoid necrosis masses are devoid of reactivity .

In cases with placental infarcts we noted the persistence of reactive cells in the villi by necrosis, with the absence of the trophoblast .

The placenta with pathological senescence is noted an increased number of cells reactive to vimentin .

Fibrinoid change and calcification areas lacked reactivity . Immunoreaction for vimentin showed dilated vessels in terminal villi, with the possibility of appreciating the membrane thickness sincitio capillaris .

CD34 is a marker used to identify endothelial cells in the placenta and thereby to study placental vascular changes during pregnancy.

The cases included in the present study we noted the presence of reactivity for CD34 in the endothelium of vessels of various sizes, in the absence of reactivity of other structures as trophoblastic and amniotic epithelium, mesenchymal cells, fibrin and fibrinoid necrosis (Fig.14,15,16,17).

Small caliber vessels marking terminal villi showed in the group from malformed placental vessels more dilated issues,with greater congestion, taking form of pseudoangiomatosis and "corangioza" .

In other cases, we noticed an increasing number of vessels at the level of villi, but this were reduced size .

In both groups of placentas studies we noticed the tendency of peripheral arrangement in villosity axis in mature placentas.

Muscle specific actin was identified using prediluted antibody Anti-Human Muscle Actin in the vessel wall myocytes from the villosital trunks of all cases of placenta analyzed and in perivascular cells arranged as sleeves around these vessels .In the absence of reactivity in the endothelial cells and trophoblastic epithelium cells.

We have not detected significant differences in marker expression between the group of placentas from malformed and control group.

In placentas with ischemic changes we noticed aspects of vessel wall hyperplasia of myocytes from the villosital trunks, with a significant reduction in the vascular lumen .

Cell layer reactive for actin are thinner near villi, trunks order II and III, because the terminal villi are poor represented in the vascular walls .

We also noticed the presence of reactive cells for muscle actin outside the vessels, cells belonging to the extra vascular contractile system (Fig.18,19,20,21).

Ki-67: MIB1 antibody (anti-Ki-67) identified the reactive nuclei in 8 from the total placentas from malformed and in two placentas in the control group, with a greater number of reactive nuclei in early placentas (Fig. 22,23,24,25)

We haven't noticed significant expression between the group of placentas from malformed and control group.

Amniotic epithelium was consistently negative for the proliferation marker.

Positive nuclei were identified in the "citotrophoblast" cell compartment and in the villosity axis (in outbreaks of hematopoiesis and in isolated stromal cells)

In all cases analyzed sincitio-trophooblast nuclei were deprived of reactivity .

Rare positive nuclei were identified in villi on the of degeneration, constrained in masses of fibrinoid necrosis.

We noticed a large number of nuclei positive for Ki-67 in cases of placentas with pathological senescence changes, with agglutinated villi, symphysis , and with the condensation of the stromae .

Alpha-fetoprotein: Anti-alpha FP antibody highlighted the presence of the brown colour of the final product of the reaction in placentas from malformed foetuses in the vessels of the villositary trunks and terminal villosities, with the appearance of a homogeneous intravascular mass or reticulated or as a complete or partial marking of the vessels' circumference (Fig.26,27,28,29), we noted weak positive reaction in the extra-villositary trophoblast.

In one case out of the studied group with hyperplasia of the syncytial buds and picnotic appearance of some syncytiotrophoblast nuclei we noticed a relatively wide positive reaction within the villi in the cases with lesions of villitis and degenerative-necrotic changes of the villosities we noticed the presence of the brown coloured final product of reaction, focally in the villositary axis and in the intervillous space.

DISCUSSION OF THE RESULTS OF THE IMMUNOHISTOCHEMICAL STUDY

Through the precision markers such as monoclonal antibodies deep structural changes were revealed in the placental architecture either in

AE1/AE3 identifies a cocktail of cytokeratines including CK8. Presently there are studies showing that within the first-trimester placenta as well as on term placentas there are a series of fibroblastic / miofibroblastic-like stromal cells expressing CK 8 and 18.

On the other hand, cytokeratines were seen as markers of trophoblast differentiation.

So caution should be exercised in IHC identification of cells isolated from placenta.

According to Haigh et al., 1999, the trophoblast must be identified by CK7 expression and not to other CK, an aspect supported by our results.

The functional significance of CK expression in mesenchymal cells is to be determined.

Regarding vimentine, literature data show that in the chorionic villi of the 1st and 3rd trimester the expression of vimentine is missing, becoming positive in term placenta.

Based on these data it is estimated that vimentine and collagen type IV is indicative of fetal maturation .

Experimental studies have noticed in the placenta the expression of vimentine in the amniotic epithelial cells (the coexpress CK and vimentine) , unacknowledged aspect of our results, showing no reactivity for vimentine in the amniotic epithelial cells under internal positive control (positive fusiforme cells from the subcorial conjunctive).

Positive reaction for vimentine is also reported in the myocytes media from the 1st order villositary tunks and in the vascular wall cells and cells arranged in sleeves around the great vessels of chorionic plate and stem villi.

These perivascular cells reactive for vimentine coexpress actin and contest which indicates that it could play a role in villous contractility and in the modelling of the intervillous space, with effects on maternal and foetal circulation .

Along with von Willebrand factor, CD 36, CD 44 and CD 31, CD 34 is a marker of vascular endothelium of the placenta.

There are studies showing modification in placental vascular structures in various stress conditions.

These studies show changes in vessel diameter and of the percentage of vessels with perivascular cells in conditions of low ambient oxygen.

These studies show that under lower oxygen pressure vessels are dilated and have fewer perivascular cells, cells that would allow the remodelling of these vessels under stress .

The immunoreactions for the muscle actin indicate continuity of the contractile function along the vessels in the human placenta .

In the human placenta there is a contractile vascular system of the foetal vessels and a secondary extravascular contractile system, consisting of long fusiforme cells, with fine cell processes which make contact with neighbouring cells and with which these cells are inserted into the trophoblastic basement membrane.

This extravascular contractile system is located in the chorionic plate and is disposed longitudinally adjacent to the foetal blood vessels from the stem villi where they form the contractile perivascular sleeves, reactive for vimentine, actin, myosin.

The marginal sinus of the intervillous space is separated from basal and chorionic plate by a layer of cells that express vimentine, desmin, actin and myosin.

This muscular ring is continuous with the media of the uteroplacentary veins that open into the marginal sinus.

Muscle cells are separated from the intervillous space through a layer of endothelial cells that is continuous with the maternal endothelium of the uteroplacentary marginal veins.

The functional importance of this muscular ring is unknown

Studies in experimental models suggest that placental growth is achieved at the expense of the cytotrophoblast proliferation so that in the placenta in early pregnancy an increased number of PCNA cells - and less Ki-67-positive trophoblast columns, are identified, particularly in the proximal portion of columns adjacent to the anchoring villi.

Ki-67 reactive cells are more numerous in the cytotrophoblast from the chorial plate in early and middle pregnancy

The number of cells with reactivity for markers of proliferation gradually decreases with age of the pregnancy this remarkable decrease in the marking index towards the term is related to a decrease of the number of Langhans cells because the rate of division in the Langans layer remains almost constant during pregnancy, in pre-eclampsia there is an increase in the rate of the trophoblastic proliferation, a possible explanation being that the syncytiotrophoblast inhuria would lead to respiratory hyperplasia of the cytotrophoblast

Chorionic villi contain cytotrophoblastic reactive cells throughout gestation, while the extravillous cytotrophoblast invading the spiral arteries shows reactivity for PCNA, but not for Ki-67

These data show that early placenta is characterized by increased proliferative activity of cytotrophoblastic cells .

Several recent studies show that there are no significant differences in PCNA marking index in placentas from trisomy 21 placentas compared to the control group (Qureshi et al., 1997).

Other studies show low Ki-67 positive nuclei in trophoblast and stromal cells in foetal intrauterine growth restriction due to abnormal circulation through the umbilical arteries, along with a reduction in vessel density and an increase in stromal cells expressing smooth muscle actin.

An increased number of cytotrophoblastic cells in placentas from pathological pregnancies were noticed (Alsta, 1996), something supported by our results showing an increased number of nuclei, cytotrophoblastic cells reactives in the placenta with pathological senescence.

The literature refers to the relationship between alpha-FP levels in maternal blood or umbilical cord and various changes of the foetus or placenta.

Data on immunohistochemical expression of alpha-FP are scarce if not absent.

There are data that show high levels of maternal alpha-FP even in preeclampsia.

One case with unexplained high levels of alpha-FP in the cord, without any birth defects, correlated with aspects of placental infarction, intervillous network of fibrin, the presence of intervillous trophoblast and cyst formation in the septum, was reported .

It appears that increased serum levels of maternal alpha-FP in the second trimester of pregnancy correlates with a poor prognosis, with the possibility of premature birth, the restriction of intrauterine growth or foetal death; increased alpha-FP appears to be determined by ischemia with subsequent stimulation of angiogenesis .

CONCLUSIONS

Immunohistochemical study shows a low degree of placental insufficiency, medium, or severe depending on the pathological changes associated placenta. . Placental pathological changes reported in our study were: villitis, inter and perivillitis, calcification, fibrinoid bridges, massive deposits of fibrinoid material,avascular villi, ischemic and chronic changes, acute and chronic infarcts, intravillosital thrombi, corangiosis, common anuclei red blood cells, sometimes outbreaks of hematopoesis, fibrinoid necrosis. All these changes translate a feto-placental pathology .A part of the listed changes sugerate that hypoxia seen as an important factor in the emergence of developmental disorders and prematurity. In our study the presence of numerous necrotic areas show that the intervention of the hypoxic mechanism is particularly important for the edification of the embryo. The use of monoclonal antibodies to express the amniotic citotrophoblastic epithelial markers or immunohistochemical markers of mesenchymal encourage the possibility to placental pre-born study, that could be in some cases suspected of developmental disorders. Use of monoclonal antibodies such as Ki 67, as a factor of expression for markers of trophoblastic reactivity of the cytoskeleton, such as citokeratines and epithelial markers like CD 34 vimentin revealed aberrant structural angio-architectural changes that translate later into modifications of placental development and other changes such as prematurity. The use of the immunohistochemical actin marker, muscle specific, revealed the presence of the myocytes in both the vascular wall of villosital trunks, and also in perivascular spaces situated away from the vessel, which suggests the presence of extravascular contractile system probably responsible for the placental with the cancellation of placental vascular lumen and production of hypoxia.

Extravascular contractile system emphasize that our study is subject to present and future.

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