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ABNORMAL DIFFERENTIATION OF THE CHORION AND ITS RELEVANCE TO THE DEVELOPMENT OF THE CONCEPTUS*

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Abstract

Chromosome investigations were carried out in 50 cases with pathological aberration of the conceptus. The majority of chorionic biopsies were analysed between the 10th and 11th weeks of pregnancy. A normal karyotype was diagnosed in 37 probes , 13 showed different types of chromosome aberrations.

The morphology of the chorionic villous tree revealed a greater number of alterations in cases with abnormal karyotypes. The same distribution pattern could be observed in histological investigations.

The frequencies of degenerative changes of the villi were the same in both groups. In 4 cases , the fate of the pregnancy could be predicted by the kind and degree of alterations in the chorion.

Introduction

The analysis of extraembryonic tissues of chorionic villi has created a new possibility of diagnosing pregnancies during the first three gestational months.

The tissue-samples enable diagnostics on different gene levels ; the gene product , the chromosome , and the DNA. Among these , ascertainment or exclusion of chromosome abnormalities is the dominating group. Another investigation is given in cases with abnormal development of the conceptus in the first weeks of ontogenesis by ultrasonography. Apart from the

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ultrasound-biometry of the conceptus , morophologic changes of embryo and extraembryonic tissues are taken into account (3-7).

The results of the cytogenetic investigations are usually received after 3 to 8 days.

In 5 to 10% of analyses in current pregnancies no definite results can be achieved after chorionic villi sampling. Therfore a second complimentary chromosome analysis has to be performed. Usually this is done by cultivation of amniotic fluid cells or fetal lmphocytes in the 16th to 20th gestational week.

The necessity of more than one chromosome analysis in current pregnancies is either given by a pathologic karyotype in extraembryonic cells, of which the importance for the further development of the embryo is uncertain , or it is given by the analysis of single pathologic cells which might present a chromosome mosaic and might be of no importance if they were restricted to the extraembryonic parts of the conceptus.

In cases of an increased genetic risk the genetic counselling of the parents is offered before carrying out any prenatal investigations (8).

If the first ultrasonographic screening reveals an abnormal conceptus; cytogenetic analyses are performed before genetic counselling , so that a more precise prognosis can be made.

Especially in these abnormal pregnancies enough information has to be gathered within a few days. Therefore not only the karyotype of the conceptus but also the developmental changes of the chorion compared to the week of gestation , morphologic changes of the chorionic villous tree and degenerative alterations are taken into account.

In the following , a number of cases showing an abnormal developmental course in early pregnancy are summarized to demonstrate the most important factors which have to be regarded as determinative for the further developmental alterations of the embryo.

Material and methods

Chromosome analyses were carried out on 50 pregnancies showing abnormal ultrasonographic screening.

The mean gestational age was 10/5. The earliest case was at the 8th , the latest from the 15th (15/3) weeks.

The mean maternal age at pregnancy was increased (X = 32.4 years) compared to the average population (X = 28.5 years) , but showed a rather wide spread from 22 to 45 years. The number of previous abortions was increased for the women , ranging from 1 to 7 as a maximum. One woman has had a child with a chromosome syndrome (trisomy 18) in a previous pregnancy , one women had a child with a monogenic inherited defect.

Treatment of the chorionic biopsy and morphologic analyses Biopsy probes gained by chorionic villi sampling (CVS) or taken from placenta material of induced abortions when a missing embryo was diagnosed , were transmitted to a sterile Petri dish containing cell culture medium.
Blood coagles and decidua tissue were separated.

The non-fixed material was investigated by a divert - microscope at magnification 25 and 100X. Four main groups of criteria were concerned , each of them subdivided into different specific features :

I Vascularisation
- reduced
- protracted
- unequal development
- missing

II Branching of the villous tree
- reduced
- unequal distribution
- increased in terminal regions
- missing

III Development of sprouts
- reduced
- unequal distribution
- missing

IV Structure of the villous tree
- changed ratio between epithelium and stroma
- varying diameter of the trophoblast
- edematous swelling of stroma

- infiltration of abnormal cells or cell-derivaies into the stroma
- Pathologic structure of blood vessels.

This model had been developed by the previous investigation of 250 chorionic biopsies , both with normal and abnormal differentiation of the villus tree (4).

Vital dyes are those substances , which can be added to the non-fixed material , giving a different staining pattern in normal vital cells , in cells with rduced vitality and in dead cells. In our investigation , this staining was performed with Acridine-Orange , which gives very specific staining differences for cells with change in vitality.

I Normal vital cells
Incorporation of only a small amount of Acridine-Orange molecules:
Green fluorescence of cytoplasm and nucleus.

II Sightly reduced vitality of the cell leading to an increased permeability of the cell membrane:
Yellow-green fluorescence of cytoplasm.

III Profoundly reduced vitality of cells:
Yellow-orange fluorescence of the whole cell.

IV Dying and necrotic cells:
Uptake of the dye uninhibited , leading to a red-brown colour of the cells.

The final concentration of the dye was 0,0005% , the time of incubation was 5' at 37^0 C. After staining the dye was removed and 1.5 ml of phosphate buffer (pH 6.9) was added to the probe.

The analysis was then carried out by fluorescence microscopy.

The probes were prepared according to the standard techniques and the slides were stained by hematoxylin-eosin and besides by immuno-peroxidase dye (4).

In all cases where the morphologic investigation and the vitality control revealed a normal vitality of the chorionic biopsy , in vivo occuring mitoses from the cytotrophoblast were gained by direct preparation of this cell-layer (5). When the vitality of the probe was obviously reduced , long term cell cultures were set up from small cuts of the tissue.

All chromosome preparations (C-metaphases) were stained by the

acridine derivative quinacrine-mustard , which induces a different intensive fluorescence in At- and GC-enriched segments of the chromosome , thus enabling the identification of the single chromosome and its intrachromosomal structure.

Results

37 out of 50 cases revealed a normal karyotype. 13 showed various chromosome aberrations :

A. Polyploidy
Karyotype:
69,XXX
69,XXY
92,XXYY

B. Aneuploidy
Karyotype:
47,Xy,+6
47,XX,+9
47,XX,+16
47,XX,+18 (2 cases)
47,XX,+21
47,XX,+22
45,X (2 cases)

C. Structural aberration
Karyotype:
46,XY,dup (9p)

Comparing pregnancies with normal and pathologic karyotype no significant differences on the basis of T-test could be observed concerning maternal age , which was $\bar{X}$= 3.18 years (22-43 years) for conceptuses with normal karyotype and $\bar{X}$= 34.0 years (23-45 years) in pregnancies with chromosome abnormalities.

Regarding the frequency of abortions in previous pregnancies of the women , no difference were found between the two groups.

The mean time of CVS for cytogenetic diagnoses was the 10th gestational week. Analysing the group of cases with a normal karyotype , the

mean week of gestations was 10/4 , varying from 8/0 to 15/0.

In the group with pathologic karyotype the mean value was almost the same with $\overline{X}$ = 10/5 , ranging from 9/3 to 15/3. Thus , in our cases with normal and abnormal karyotypes , pathologic alterations of the conceptus at almost the same phase of ontogenesis were observed.

All cases revealed an abnormal morphology of the chorionic villi. The number of villi was reduced , the branching of the villous tree was irregulr and often reduced. The rami tended to be croweded in the terminal regions. The villi varied in their diameter even within the same case from filiformed to clubformed. These caliber differences occured more often in the peripheral than in the proximal villi. The vasculrisation was in the majority of cases not appropriate to the gestational week. Mostly , it was reduced or even missing , in single cases prevailing in parts of the chorion (Fig. 1).

The number of sprouts was reduced and mainly irregularly distributed over the villous tree. The surface of the trophoblast epithelium revealed a decreased number of microvilli and was often irregularly notched or wrinkled.

Comparing cases with normal and pathologic karyotype , those with chromosome aberrations had on an average a greater number of different types of morphologic alterations of the chorion than those with normal chromosomes.

The vitality of chorion samples was normal in 50% of the cases in spite of showing a pathologic morphology of the villous tree. This group comprised cases with normal as well as those with a pathologic karyotype.

In 35% of the biopsies , a slightly reduced vitality of the cells visible by an increased uptake of Acridine-Orange in cytoplasm and nucleus , was observed. The altered cell groups were found irregularly scattered over the chorion and they were always restricted to peripheral rami and sprouts. Only in 15% of the investigated cases , peripheral parts of the villous trees revealed a greater amount of dead cells , but the stem villi were unchanged in vitality as well as the stroma of the proximal rami. In these cases direct preparations of mitoses from the cytotrophoblast were no longer practicable but chromosome investigations could be performed by long term cell cultures.

Biopsies with normal vitality and those with reduced vitality showed an equal amount of cases with normal and pathologic karyotype.

The revealed significant differences in the number and extent of

alterations compared to the normal differentiation of the chorionic villi in the respective gestational week (Fig. 2). The combination of alterations in the single probe will be given in the following by detailed description of two cases.

Trisomy-18 (11th week of gestation) in three different types of villi were observed. I , there were villi with an almost normal stucture and only slightly reduced vascularisation ; II , there were villi with plump and compact appearance without vascularisation with smooth surface of the trophoblast epithleium ; III , the last group consisted of villi with rudimentary vascularisation , the stroma being cell-rich and the trophoblast epithlium irregular. The trophoblast epithelium itself was different in its height and number of cell-layers. Some villi showed foci of calcification.

Trisomy 22 (11th week of gestation) in three types of villi were analysed. The majority was vascular with retarded development. Some villi showed reduced vascularisation with the blood vessels being extremely narrow in lumen. A small number of villi had delated terminal regions with an excess of large blood vessels. The epithelium was uneven with partial invaginations into the stroma , thus developing pseudo-cysts.

Secondary changes of the chorionic villus tree could be divided into 4 main groups:

I deposition of fibrin on and in the villi
II Hydropic swelling of chorionic villi
III Detachement of the trophoblast epithelium
IV Reduction of the vitality of the cells

Degenerative changes of the chorion were documented by the combination of morphologic , histologic , and vitality analyses. No preference as to early or late pregnancy age was observed.

Cases with pathologic karyotype showed degenerative alterations in the same frequency and degree of severity as those with a normal chromosome constitution.

In the whole , 38% of the biopsies revealed degenerative alterations of the chorion (Fig. 3).

Developmental differences in the abnormal chorionic tissue in regarding different cases with the same chromosome abnormality (trisomy 18 ,

monosomy X) , it was striking to notice that the amount and the expression of pathologic changes in the placenta differed distinctly. In parts , this might be caused by chromosome mosaics , which for example amont to alomst 50% in monosomy X cases.

It was not always possible , to proove the existence of the second cell-line , perhaps because of an irregular distribution in the placenta or even between trophoblast epithelium and stroma.

Besides , other factors might have enabled the almost normal differentiation of the villi in certain regions of the placenta , as it could be observed in our case of trisomy 18. Villi with less retarded diferentiation were not restricted to the center of the chorion frondosum.

If it is possible , as in 3 of our cases with chromosome aberration to get 2 or even more biopsies for investigation over a period of more than two weeks , information can be obtained on the lonitudinal changes in the pathologic villous tree. In two of our cases it could be stated that the amount of less altered villi was rising within the time of investigation , in the third one alterations were changing to the worse.

In cases of extremely abnormal development of the chorion at an early stage of gestation a fatal course of the pregnancy had to be predicted , althought , by sonography , the embryo appeared almost normal at the time of the first investigation (trisomy 9 , trisomy 22 , dup9p).

Discussion

Our investigations on early pregnancies with an abnormal course showed that about one third was due to chromosome abnormality of the conceptus. The degree of developmental disturbances depended on the karyotype , chromosome constitution and type of abnormality. Severe impairment during the differentiation of the chorion lead to secondary changes , such as hydropic swelling of the villi or terminal dilatations of blood vessels , leading intrauterine death of the embryo.

In two cases , hydropic swellig of single chrionic villi , lead to such excessive dilatation of the tissue, that false diagnosis of a twin pregnancy was given during ultrasonographic investigation suggesting that each case was an empty amniotic sac , one triploidy and the other trisomy 21.

The morphologic change in the extraembryonic tissues with chromosome aberration reveal a pattern of frequent and rare symptoms as it is known from embryos , fetuses , and children with chromosome syndromes (2 , 3 , 4 , 6 , 7). Our intension is to find out the typical combination of symptoms characterising the individual chromosome abnormality , as we did it during the last 30 years in patients with chromosome syndromes.

Another problem , which has arisen during the last 5 years is that growing number of chromosome mosaics is found when ivestigating embryonic and extraembryonic tissues of early pregnancies (1).

When chromosome investigation was only possible by long term cell cultures it was assumed that the majority of pathologic clones and single cells was caused by in vitro alterations of the cells. But nowadays those aberrant cells were also diagnosed in short term cultures as well as in direct preparations of mitoses (1).

The significance of these cells to the development of the conceptus is still unknown. As the majority of pregnancies with single patholgic cells in the extraembryonic tissues shows a normal course , it has been supposed , that there is a selectin against single cells with a pathologic karyotype during ontogenesis.

Experiments are now carried out in our investigation group to get further information about these peculiarities in early pregnancies.

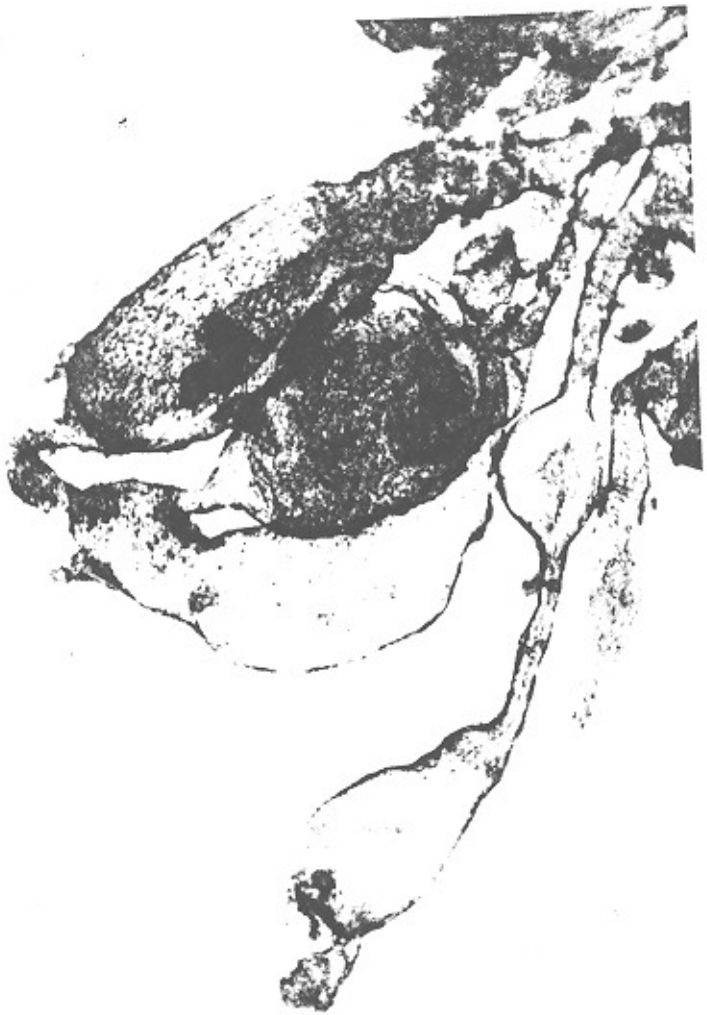

Fig. 1- Partial hydropic swelling of the chorionic villous tree (11th gestational week).

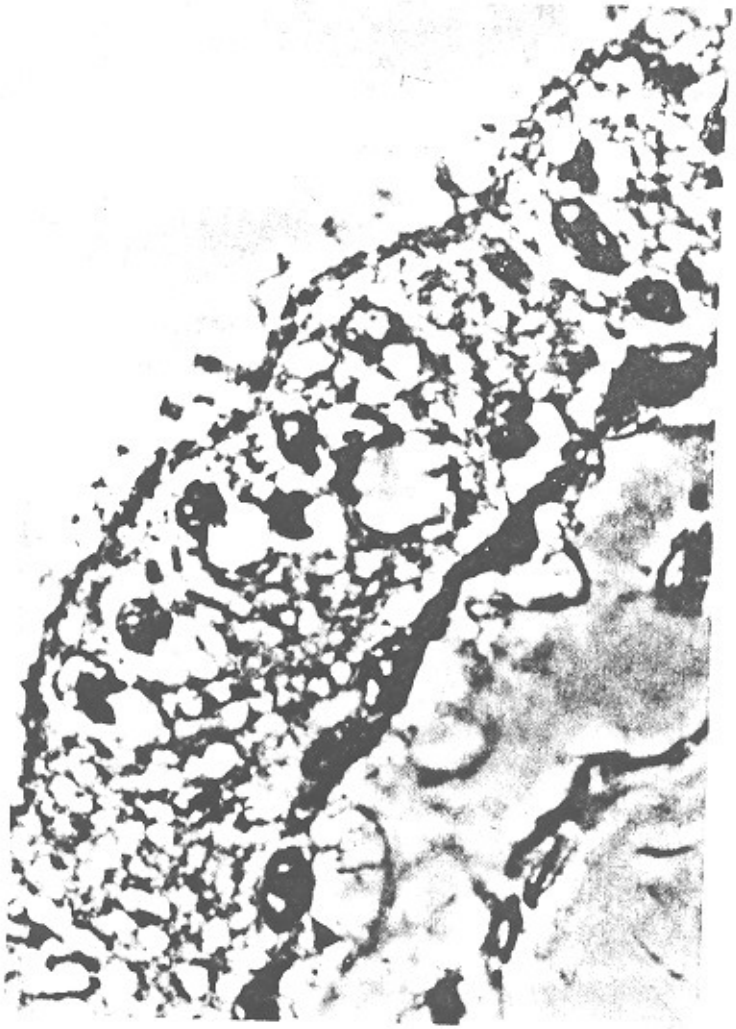

Fig. 2- Changes of the trophoblast epithelium (8th gestational week). CL- Increased height of epithelium and decreased number of nuclei.

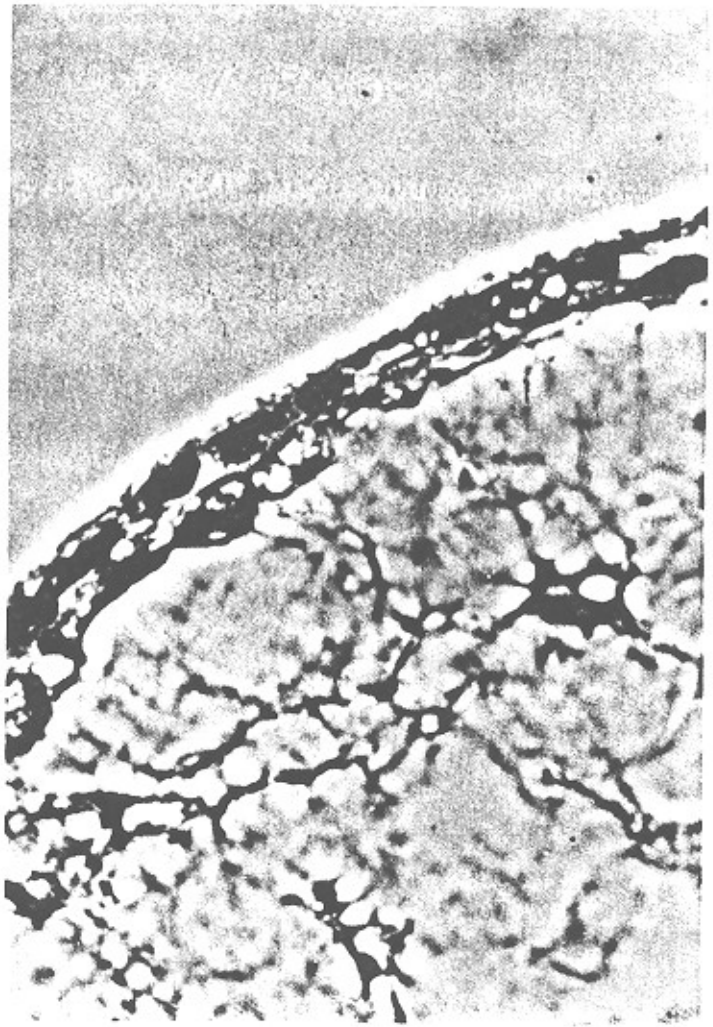

Fig. 2- Changes of the trophoblast epithelium (8th gestational week). b. Decreased height of epithelium , reduced number of microvilli.

Fig. 3- Degenerative alterations of chorionic villi (11th gestational week). Foci of calcificantion in the stroma of the villi.

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