Amniotic fluid cell and chorionic villi culture for prenatal HLA-determination.

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HLA-determination on placental or fetal tissue are of interest in Forensic Medicin as well as in Medical Genetics.I Forensic Medicine prenatal HLA-determination of the fetus supports a paternity diagnosis. In Medical Genetics one important aspect is prenatal diagnosis of congenital adrenal hyperplasia, as there is a close linkage between the HLA-B locus and a deficiency in 21-hydroxylase. Furthermore prenatal HLA-determination is of interest in relation to transplantation immunology.

We have elaborated methods for culture of amniotic fluid cells and villus cells for HLA-determination. HLA-determination on cultured amniotic fluid cells was previously not performed in Denmark. The recent introduction of chorionic villus biopsies for prenatal diagnosis made an investigation about 4-6 weeks earlier than amniocentesis possible. As the time for a villus biopsy may have passed when the investigation is wanted it will be necessary to handle both techniques.

The material for a fetal diagnosis can be obtained during pregnancy as a villus biopsy in the 8-12 week or as fetal cells isolated from amniotic fluid after amniocentesis in the 16th week of gestation. Abortion material, whether spontaneous or induced, consists of the fetal parts of the placenta, the membranes or the fetus proper. Especi-

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ally after fetal death placental cells may show the best growth potential.

Material and methods.

Cell cultures were established on from 3 chorionic villus biopsies, 10 samples of amniotic fluid and 22 samples of villi from hydatidiform moles. The molar pregnancy is in most cases characterized by absence of a fetus, cystic swelling of the villi and 46 chromosomes originating from a duplication of the 23 paternal chromosomes. As the maternal chromosomes are missing only paternal antigenes are expressed. Finally 3 cultures of fetal fibroblasts were processed.

After amniocentesis the culture of amniotic fluid cells followed the routine procedure at the lab. Briefly, the cells in 15-20 ml amniotic fluid are spun down and incubated in medium 199 or McCoy's medium supplemented with 10% fetal calf serum and 10% pooled human serum. Usually the cells are subcultured on day 10-14 and the secondary cultures confluent after 1-3 days. When a sufficient number of cells was obtained in the secondary cultures the cells were trypsinized and transferred for final growth into microchambers used for typing as described for villus cells below.

Placental parts of fetal origin are obtained prenatally by villus biopsy or after abortion by dissection of the placenta under the microscope. In both situations the initial important step is isolation of fetal villi and removal of blood clots or tissue of maternal origin. The following enzymatic disaggregation of the tissue by collagenase removes the trophoblast layer on the surface of the villi and exposes the stromal cells. Villus trophoblast cells do not express HLA-antigens and it is thus intended to obtain cultures exclusively of stromal cells. Furthermore untreated villi in culture show primary outgrowth of slowly dividing trophoblast cells that later on

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are replaced by stromal cells. When trophoblast cells are removed before the villi are set up in culture the time for HLA-determination is reduced considerably. By centrifugation the villus stems are concentrated in the pellet and set up for culture.

Primary cultures are established in Chang medium in T 25 flasks. Chang medium, which is supplemented with several growth factors and hormones, was originally designed for improved growth of amniotic fluid cells, but applied to chorionic villi the time in culture is reduced too. Outgrowth of fibroblastlike, stromal cells is observed around the tissue explant within 1-3 days. On the 3rd day the medium is replaced by DMEM with 20% fetal calf serum and 10% pooled human serum. Secondary cultures are usually established on day 4-5 and confluent on day 8-10. HLA-determination on cells in suspension immediately after harvest with trypsin revealed a very high percentage of dead cells - often about 40%. This was reduced to less than 10% by final culture in microchambers used for HLA-determination.

After harvest 200.000 cells are suspended in 1.5 ml medium and 15 mikroliters of this suspension are placed in each well of 2-4 microchambers used for HLA-determination. When the cells are attached to the bottom of the wells 5 ml of medium are added and the microchambers incubated for 24-48 hours to obtain a sufficient number of cells. The incubation period reduced the background of dead cells due to the preceeding trypsinization and increased the number of cells expressing HLA.

Conclusion.

Isolation and culture of villus stromal cells by the present techniques has improved the growth rate of cells expressing the fetal HLA-haplotypes. The final culture of amniotic fluid cells and villus stromal cells in the microchambers used directly for HLA-determination reduced

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the background of dead cells observed immediately after trypsinization. The culture of villus stromal cells provides a prenatal HLA-determination about 4 weeks earlier than it is possible to obtain by amniocentesis.
It is now possible to obtain the proper material for HLA-determination, whether it is performed on amniotic fluid or villus cells and whether the purpose is based on Forensic or Medical Genetics.

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