Original Investigations

Antenatal Sex Determination in Blood from Pregnant Women

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Summary. Reported results concern Y-chromatin of lymphocytes and granulocytes in the peripheral blood of pregnant women. In women who later delivered male children, a mean of 3.75% Y-chromatin was found in lymphocytes. Even after investigation of paternal Y-chromatin, the rate of false diagnoses (14%) in prenatal sex diagnosis could be reduced only moderately.

In early pregnancy lymphoid cells with a Y-chromatin could be first traced only in the 8th week; granulocytes showing a Y-chromatin did not appear until the 9th week.

It is known that fetal leucocytes are able to penetrate through the placenta into maternal circulation. In order to determine the number of fetal cells, Walknowska et al. (1969) and de Grouchy and Trebuchet (1971) examined lymphocyte cultures in mitosis from maternal blood. Cells from male fetuses are normally discernible from maternal cells by the Y-chromatin. De la Chapelle and Schröder (1971), Schröder and De la Chapelle (1972), Rogall and Zinser (1973), Schröder et al. (1974) and Grosset et al. (1974) chose interphase nuclei for their investigations. The material for their studies dated back only to the 14th week of gestation. None of the fathers were included in the investigations.

The present study aimed at testing the reliability of antenatal sex determination by means of fetal interphase nuclei in maternal circulation. Paternal leucocytes were taken as a comparative parameter because of the variability of Y-chromatin and other interphase fluorescence markers. Furthermore, we tried to determine the earliest time in pregnancy at which fetal cells can be detected in maternal circulation.
Material and Methods

One hundred human females were tested. In order to avoid inaccurate results, which may be caused by persisting lymphocytes, those women who had given birth to male children more recently than 2 years before were excluded from our study.

In paternal blood Y-chromatin containing cells were classified into 4 categories according to size and brightness of fluorescence. In this way we intended to distinguish possible fluorescence of autosomal chromosome regions from the Y-chromatin.

The frequency of Y-chromatin containing cells in paternal blood smears varies according to staining conditions and size of the individual Y-chromatin. In order to guarantee standard conditions, blood smears of the mothers and the fathers were stained simultaneously, and the number of lymphocytes and granulocytes necessary to count 100 Y-chromatin bodies in the paternal blood was calculated. This number was our standard for counting maternal cells. Thus we could assume to obtain an absolute percentage of fetal lymphocytes and granulocytes in the blood of pregnant women. Lymphocytes and granulocytes were evaluated separately (see Table 2).

Our earliest case was in the 7th week of gestation. In the group of women from the 7th to 12th weeks of pregnancy we carried out some follow-up studies.

The examinations were performed in the following way: Smears of maternal and paternal blood were air dried for ½ to 12 hrs. Then the slides were fixed for 3 min with acetic acid:methanol 1:3 and were air dried. They were subsequently stained with quinacrine dihydrochloride (0.5 g of quinacrine dihydrochloride in 100 ml of 0.02 nHCL), rinsed in Sørensen's buffer, pH 4, mounted with buffer and sealed with nail polish.

Results

In the screening of paternal blood smears we found a mean frequency of 79.8% Y-chromatin containing lymphocytes, with a range from 52 to 96%. Granulocytes rendered a mean of 72% with a range from 50 to 100%.

The results from the pregnant women are shown in Table 1. Among 76 maternal blood smears between the 4th and 10th months of pregnancy we found Y-chromatin containing cells in 46 cases. In the remaining 30 cases no definite Y-chromatin containing cells could be traced. In the 46 Y-chromatin positive cases the mothers gave birth to female infants in 7 cases. Q-banding patterns in 3 of them revealed extremely bright fluorescence at the centromere of chromosome No. 3. In addition, one of these cases showed brightly fluorescent satellites in chromosome No. 13.

Among the 30 maternal blood smears in which no Y-chromatin was identified, one mother delivered a male child. In this case we indeed found fluorescent particles, but they were misinterpreted as autosomal fluorescence, although the paternal Y-chromatin in this case was extremely small. When the infant's blood was screened it rendered small but unequivocal Y-bodies.

A similar situation is found among the 24 cases from the 8th to 12th weeks, where in 4 cases female and in 2 cases male diagnoses were missed.

In 5 of these early cases with Y-chromatin containing cells follow-up studies were performed (see Table 3). From Table 2 it is obvious that the frequency of Y-chromatin containing cells is slightly higher at the end of pregnancy than in the early months. There is, however, a vast individual range. In maternal circulation, fetal lymphocytes are no more frequent than are fetal granulocytes during the complete course of the pregnancy. Only in the 3rd month is there a clear preponderance of lymphocytes.