Increase in the amount of fetal lymphocytes in maternal blood during pregnancy

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SUMMARY The presence of male fetal lymphocytes in the maternal blood of 18 pregnant women (15 primigravidae and three who had had previous pregnancies) was analysed by counting quinacrine positive Y bodies in interphases or Y chromosomes in metaphases.

Counts were also performed on a control population of ten young women who had never been pregnant and on another control population of seven men to test the efficiency of the staining technique used.

After completion of the calculations, comparison of the results with the sex of the newborn babies showed a correct sex prediction of 83% by interphase scoring and of 89% by metaphase scoring.

The lymphocyte transfer from fetus to mother has already started by the tenth week of pregnancy, and the percentage of fetal cells present in the bloodstream of a pregnant woman increases with the duration of the pregnancy.

The transfer of blood cells between mother and fetus in man is an established fact. Schröder estimated, using immunofluorescent techniques, that one fetal erythrocyte is present for every 50 000 maternal erythrocytes in maternal blood. During the first trimester of pregnancy this transfer from the fetus to the mother takes place in only 10% of mothers; later on in the pregnancy this increases to 40% of the mothers. These cells are viable for about 200 days if the blood groups of mother and child are compatible.

The presence of maternal erythrocytes in fetal blood is described by different authors; these erythrocytes represent between 1 to 10% of the total fetal blood volume but disappear quickly after birth (4 days). The transfer of lymphocytes from the fetus to the mother was studied by analysing the presence of male cells in maternal blood either by counting the number of acrocentrics without banding on metaphase cells or by scanning Y positive interphases after Q banding. The average frequency of fetal lymphocytes present in maternal blood was shown to be one fetal lymphocyte in 500 to 1000 maternal lymphocytes. These cells are sometimes present 3 to 5 years after delivery and may thus interfere with fetal cells of following pregnancies. The presence of maternal lymphocytes in fetal blood, however, seems to be relatively rare (about 0.07% maternal lymphocytes in 1/10 of the children).

Many problems related to this cell transfer remain unsolved. How does transfer occur? When does this transfer start? What about immunological tolerance? This work presents a transverse analysis (from 6 to 40 weeks’ gestation) and a tentative longitudinal (two blood samples per pregnancy) analysis of the frequency of fetal lymphocytes in the blood of primigravidae by quinacrine staining of the Y chromosome in interphase and metaphase cells.

Material and methods

SELECTION OF BLOOD SAMPLES Peripheral blood was collected from three groups: (1) a test population made up of 18 pregnant women who had not had a male child before and whose history of spontaneous abortions was checked. Gestation time was expressed in weeks after last menstruation. Each woman was examined once, twice, or three times; (2) a control population made up of ten young women who had never been pregnant; and (3) another control population made up of...
seven men. These cells were needed to estimate the exact efficiency of the Y detecting technique used.

DETECTION OF Y CHROMOSOMES
Peripheral lymphocytes were cultured for 48 hours and prepared by the method of Moorhead et al. The slides with cells collected from these cultures were hypertonically treated in a Q buffer (citric acid 0.05 mol/l, Na₂HPO₄ 0.1 mol/l, pH 4.1) for 7 minutes and stained for 8 minutes in a quinacrine dihydrochloride solution (100 μg/ml) prepared in the same Q buffer. After soaking again for 7 minutes in the Q buffer, the slides were mounted in the Q buffer and observed by fluorescence microscopy (Zeiss microscope with a mercury lamp illumination excitation filter: BP 450–490; barrier filter: LP 520). The test and the two control populations were analysed separately.

For each of the test and two control populations, the number of Y bodies was counted in 5000 interphase nuclei and the presence of a Y chromosome in 1000 metaphase cells. The sex of the newborns was unknown until all calculations were completed. For each of the control population, 100 metaphases and 300 interphases were examined.

Results and discussion

PRESENTATION OF INDIVIDUAL RESULTS (FALSE POSITIVES AND FALSE NEGATIVES)
The results are shown in tables 1, 2, and 3. Table 1 shows that with the number of interphases and metaphases counted it is possible to see clear differences between pregnancies carrying male and female fetuses. However, false positives (case No 18 and 22) were scored for interphases in two of 18 pregnancies, probably because of unknown earlier pregnancies or through misinterpretation. False negatives were scored for the interphases of one pregnancy which resulted in a male (No 14) and for the metaphases of two which resulted in a male (No 19 and 59). These errors cannot have resulted from non-fluorescent Y chromosomes since they were all detectable either in metaphase or in interphase.

<table>
<thead>
<tr>
<th>Code No</th>
<th>Age of mother (yr)</th>
<th>Earlier pregnancies</th>
<th>Gestation time at sampling</th>
<th>Interphases Without Y</th>
<th>With Y</th>
<th>%</th>
<th>Metaphases Without Y</th>
<th>With Y</th>
<th>%</th>
<th>Sex of child at birth</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>19</td>
<td>—</td>
<td>8</td>
<td>4999</td>
<td>1</td>
<td>0.02</td>
<td>1000</td>
<td>—</td>
<td>0</td>
<td>F</td>
</tr>
<tr>
<td>22</td>
<td>18</td>
<td>—</td>
<td>7</td>
<td>4999</td>
<td>1</td>
<td>0.02</td>
<td>1000</td>
<td>—</td>
<td>0</td>
<td>Twin FF</td>
</tr>
<tr>
<td>27</td>
<td>17</td>
<td>—</td>
<td>18</td>
<td>5000</td>
<td>0</td>
<td>0</td>
<td>630</td>
<td>—</td>
<td>0</td>
<td>F</td>
</tr>
<tr>
<td>37</td>
<td>27 1 daughter</td>
<td>16</td>
<td>5000</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>320</td>
<td>—</td>
<td>0</td>
<td>F</td>
</tr>
<tr>
<td>39</td>
<td>21</td>
<td>—</td>
<td>5000</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>983</td>
<td>—</td>
<td>0</td>
<td>F</td>
</tr>
<tr>
<td>45</td>
<td>21</td>
<td>—</td>
<td>5000</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>730</td>
<td>—</td>
<td>0</td>
<td>F</td>
</tr>
<tr>
<td>Table 1 Frequencies of Y bodies in interphases and metaphases of the test population</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>
Increase in the amount of fetal lymphocytes in maternal blood during pregnancy.

### TABLE 2 Frequencies of Y bodies in interphases and metaphases of the female control population

<table>
<thead>
<tr>
<th>Code No</th>
<th>Age (yr)</th>
<th>Interphases</th>
<th>Metaphases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Without Y</td>
<td>With Y</td>
</tr>
<tr>
<td>K 16</td>
<td>24</td>
<td>5000</td>
<td>0</td>
</tr>
<tr>
<td>K 17</td>
<td>19</td>
<td>5000</td>
<td>0</td>
</tr>
<tr>
<td>K 18</td>
<td>19</td>
<td>5000</td>
<td>0</td>
</tr>
<tr>
<td>K 19</td>
<td>19</td>
<td>5000</td>
<td>0</td>
</tr>
<tr>
<td>K 20</td>
<td>19</td>
<td>4999</td>
<td>1</td>
</tr>
<tr>
<td>K 21</td>
<td>19</td>
<td>5000</td>
<td>0</td>
</tr>
<tr>
<td>K 22</td>
<td>19</td>
<td>5000</td>
<td>0</td>
</tr>
<tr>
<td>K 23</td>
<td>19</td>
<td>5000</td>
<td>0</td>
</tr>
<tr>
<td>K 24</td>
<td>19</td>
<td>5000</td>
<td>0</td>
</tr>
<tr>
<td>K 25</td>
<td>19</td>
<td>4999</td>
<td>1</td>
</tr>
</tbody>
</table>

### TABLE 3 Frequencies of Y bodies in interphases and metaphases of the male control population

<table>
<thead>
<tr>
<th>Code No</th>
<th>Age (yr)</th>
<th>Interphases</th>
<th>Metaphases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Without Y</td>
<td>With Y</td>
</tr>
<tr>
<td>M 1</td>
<td>23</td>
<td>120</td>
<td>180</td>
</tr>
<tr>
<td>M 2</td>
<td>21</td>
<td>125</td>
<td>175</td>
</tr>
<tr>
<td>M 3</td>
<td>45</td>
<td>134</td>
<td>166</td>
</tr>
<tr>
<td>M 4</td>
<td>24</td>
<td>144</td>
<td>156</td>
</tr>
<tr>
<td>M 5</td>
<td>25</td>
<td>146</td>
<td>154</td>
</tr>
<tr>
<td>M 6</td>
<td>32</td>
<td>117</td>
<td>183</td>
</tr>
<tr>
<td>M 7</td>
<td>23</td>
<td>141</td>
<td>159</td>
</tr>
</tbody>
</table>

However, either low frequencies of fetal cells in maternal blood or insufficient amounts of cells counted may explain these errors.

Data obtained from the female control population (table 2) indicate that technique-dependent errors are extremely low; these errors result from erroneous interpretation since autosomal fluorescence was not observed in metaphases (false positives).

Blood samples from seven healthy adults were used as controls for staining. The mean percentage of interphases with detectable Y bodies was 56% (51 to 61%) and the mean percentage of metaphases with detectable Y bodies was 83% (75% to 89%). These values agree well with previous observations.

### ACCURACY OF SEX PREDICTION

Accuracy of sex prediction based on interphases only (table 4), on metaphases only (table 5), or on both together (table 6) shows that the quinacrine staining used allows an 83% correct prediction by interphase scoring and an 89% correct prediction by metaphase scoring. It is also clear that the presence or the absence of a Y chromosome in metaphase is not always confirmed by Y bodies in interphase.

It might be considered that the analysis is acceptable only when both metaphases and interphases give the same results. In that case, our prediction is about 72% accurate (13/18 F). Our percentage of correct sex predictions agrees with those found by other authors, both in metaphase counts (Walkowska et al., 83%; de Grouchy and Trébuchet, 67%; Schindler et al., 38%) and in interphase counts (Schröder and de la Chapelle, 71%; Grosset et al., 86%; Zilliacus et al., 72%). Our results were favourably influenced by the following: the increase in the number of metaphases counted (1000 instead of 500), the use of banding instead of counting non-banded acrocentrics, and the selection of primigravidae (or mothers of girls).

### TABLE 4 Exactitude of sex prediction from Y bodies in interphases

<table>
<thead>
<tr>
<th>Mothers examined</th>
<th>Sex of child at birth</th>
<th>Interphases studied</th>
<th>Total</th>
<th>% exact prediction</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>M</td>
<td>115 000</td>
<td>51</td>
<td>(0-04%)</td>
</tr>
<tr>
<td>1</td>
<td>M</td>
<td>15 000</td>
<td>0</td>
<td>(0%)</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>35 000</td>
<td>0</td>
<td>(0%)</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>20 000</td>
<td>3</td>
<td>(0-02%)</td>
</tr>
</tbody>
</table>

### TABLE 5 Exactitude of sex prediction from Y chromosomes in metaphases

<table>
<thead>
<tr>
<th>Mothers examined</th>
<th>Sex of child at birth</th>
<th>Metaphases studied</th>
<th>Total</th>
<th>% exact prediction</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>M</td>
<td>12 949</td>
<td>15</td>
<td>(0-12%)</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>310</td>
<td>0</td>
<td>(0%)</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>8163</td>
<td>0</td>
<td>(0%)</td>
</tr>
</tbody>
</table>

### TABLE 6 Accuracy of sex prediction from interphase and metaphase counts together

<table>
<thead>
<tr>
<th>Sex of child at birth</th>
<th>Interphases</th>
<th>Metaphases</th>
<th>No of mothers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>With Y body</td>
<td>Without Y body</td>
<td>With Y chromosome</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>M</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

### TRANSVERSE STUDY OF PRESENCE OF Y CHROMOSOME DURING PREGNANCY

Fig 1 shows variations in the frequency of positive Y bodies in fetal interphase cells in the blood of women pregnant with a male fetus. Fig 2 gives similar data for Y chromosomes in metaphase cells.
It is clear that both the frequency of Y bodies in interphases and of Y chromosomes in metaphases increase with gestation time.

Results obtained by other authors do not show this increase if taken separately. However, if all data obtained by other laboratories are collected, an increase of Y bodies and Y chromosomes during pregnancy is seen. We will thus consider that the number of Y-containing leucocytes increases with gestation time in women pregnant with a male fetus.

**Longitudinal study of presence of Y chromosome during pregnancy**

Because of the lack of test material, the longitudinal analysis was feasible only for interphase counts and in only seven of the women pregnant with a male fetus. Fig 3 gives the observed frequencies of Y bodies in interphase cells in two blood samples from each mother taken at different gestation times. Interpretation is difficult since the response seems to vary with gestation and mother. Moreover, some of the differences observed during one pregnancy may be the result of an insufficient amount of cells being analysed. More samples per mother are necessary to perform an accurate analysis.

**Earliest detection of male cells in peripheral blood of pregnant women**

Blood samples were taken as soon as 6 weeks after last menstruation; however, we were only able to detect positive staining Y chromosomes at the earliest in a 10 week pregnancy.

This observation would signify that sex determination is possible earlier than is mentioned in other works: the 12th week by Schindler et al; the 13th week by de Grouchy and Trébuchet; the 14th week by Grosset et al and by Walkowska et al; the 15th week by Zilliacus et al; and the 16th week by Schröder and de la Chapelle.

Moreover, although fetal lymphopoiesis is classically considered to start at the 16th week, Playfair et al showed that lymphocytes are present in the fetus at the 8th week.

**Real percentage of fetal cells present in peripheral blood of pregnant women**

The frequency of Y bodies in interphases varies between 0.02 to 0.14%. If we now take into account the fact that, with our staining technique, only between 51 to 61% of the interphases of male peripheral blood cells react positively (table 3), the real percentage of fetal lymphocytes present in the peripheral blood of a pregnant woman would vary between 0.04 and 0.23%.
Increase in the amount of fetal lymphocytes in maternal blood during pregnancy

A similar calculation for the real percentage of Y chromosomes present in metaphases of women pregnant with a male fetus shows a variation between 0·13 and 0·43%. These results agree with observations made by Schindler et al. on metaphases and by Schröder and de la Chapelle on interphases. Walkowska et al. and de Grouchy and Trébuchet, however, found higher values (0-1 to 2%). This discrepancy may be because of the identification method used, since they neither had a correction factor for staining efficiency when counting small acrocentrics. On the other hand, Schindler et al., who also chose the acrocentric counting method, had results similar to ours.

Conclusion

From the data presented, two results must be underlined. Firstly, the lymphocyte transfer from fetus to mother has already started by the 10th week of pregnancy and, secondly, the percentage of fetal cells present in the bloodstream of a pregnant woman increases with the duration of the pregnancy. This shows, therefore, that by a harmless analysis of maternal blood the karyotype of male fetuses can be analysed earlier and more readily (one week) than by amniocentesis. Nevertheless, various disadvantages must be noted: firstly, there is only a 83 to 89% correct sex prediction; secondly, analysis is possible only for primigravidae; and finally no easy distinction between mother’s and daughter’s chromosomes can be made despite the fact that the latter is theoretically feasible using lateral asymmetry or other polymorphisms.

The use of this method for routine prenatal diagnosis will therefore depend on the improve of biochemical and immunological techniques. Turunen et al. distinguished fetal lymphocytes from maternal lymphocytes by a fluorescence-activated cell sorter. Also it is possible that some mitogens might selectively stimulate fetal cells.

As far as the function of the placenta is concerned, an observed increase of fetal lymphocytes during the pregnancy may signify any of the following: an increase in the amount or size of the transfer places in the placenta, an increase in the amount of cells transferred with the fetal blood volume, or an accumulation of consecutive haemorrhages. A longitudinal study based on more samples per pregnancy would give a better description of the phenomenon.

We would also like to thank Mrs Paulus-Van Mechelen, Mrs G Plas, Mr R Wellens, and Mr F Raeymaekers for skilloful technical help.

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References


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