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. Schröder¹ 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.²⁻⁹ 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 authors¹⁰⁻¹³; these erythrocytes represent between 1 to 10% of the total fetal blood volume but disappear quickly after birth (4 days).¹³

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 cells¹⁴⁻¹⁶ or by scanning Y positive interphases after Q banding.¹⁷⁻¹⁹ 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).²⁰

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.*²¹ The slides with cells collected from these cultures were hypertonically treated in a Q buffer (citric acid 0.05 mol/l, Na₂ HPO₄ 0.1 mol/l, pH 4.1) for 7 minutes and stained for 8 minutes in a quinacrine dihydrochloride solution (100 γ /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 new-

borns 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 1 *Frequencies of Y bodies in interphases and metaphases of the test population*

Code No	Age of mother (yr)	Earlier pregnancies	Gestation time at sampling	Interphases			Metaphases			Sex of child at birth
				Without Y	With Y	%	Without Y	With Y	%	
18	19	—	8	4999	1	0.02	—	—	—	F
			18	4999	1	0.02	1000	—	0	
22	18	—	7	4999	1	0.02	1000	—	0	Twin FF
			17	5000	0	0	1000	—	0	
27	17	—	18	5000	0	0	630	—	0	F
			32	5000	0	0	1000	—	0	
37	27	1 daughter	16	5000	0	0	320	—	0	
			21	5000	0	0	500	—	0	F
39	21	—	21	5000	0	0	983	—	0	F
45	21	—	6	5000	0	0	730	—	0	F
			7	5000	0	0	1000	—	0	
3	23	—	12	5000	0	0	—	—	—	
			33	4999	1	0.02	793	3	0.38	M
4	22	—	7	5000	0	0	—	—	—	
			28	4999	1	0.02	809	1	0.12	M
			15	5000	0	0	—	—	—	
14	23	—	27	5000	0	0	834	1	0.12	M
			39	5000	0	0	1000	0	0	
17	30	3 spontaneous abortions	8	5000	0	0	—	—	—	
			19	4999	1	0.02	999	1	0.10	M
			18	5000	0	0	—	—	—	
19	27	—	34	4998	2	0.04	60	—	0	M
			10	4998	2	0.04	999	1	0.10	
23	28	—	26	4998	2	0.04	365	0	0	M
			19	4998	2	0.04	999	1	0.10	
24	23	—	28	4999	1	0.02	1000	0	0	M
			29	4997	3	0.06	600	0	0	
26	17	—	35	4996	4	0.08	998	2	0.20	M
			12	4998	2	0.02	354	0	0	
30	21	—	23	4998	2	0.02	622	1	0.16	Twin FM
			29	4995	5	0.10	999	1	0.10	
49	32	1 daughter	37	4998	2	0.02	—	—	—	M
			41	4998	2	0.02	300	—	0	
			38	4995	5	0.10	250	—	0	
59	17	—	40	4993	7	0.14	—	—	—	M
			37	4997	3	0.06	266	—	0	
64	15	—	43	4996	4	0.08	997	3	0.30	M

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

Code No	Age (yr)	Interphases			Metaphases		
		Without Y	With Y	%	Without Y	With Y	%
K 16	24	5000	0	0	889	0	0
K 17	19	5000	0	0	859	0	0
K 18	19	5000	0	0	1000	0	0
K 19	19	5000	0	0	650	0	0
K 20	19	4999	1	0.02	1000	0	0
K 21	19	5000	0	0	963	0	0
K 22	19	5000	0	0	1000	0	0
K 23	19	5000	0	0	790	0	0
K 24	19	5000	0	0	900	0	0
K 25	19	4999	1	0.02	1000	0	0

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

Code No	Age (yr)	Interphases			Metaphases		
		Without Y	With Y	%	Without Y	With Y	%
M 1	23	120	180	60	19	81	81
M 2	21	125	175	58	17	83	83
M 3	45	134	166	55	25	75	75
M 4	25	144	156	52	19	81	81
M 5	25	146	154	51	11	89	89
M 6	32	117	183	61	18	82	82
M 7	23	141	159	53	12	88	88

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.¹⁷

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

TABLE 4 Exactitude of sex prediction from Y bodies in interphases

Mothers examined	Sex of child at birth	Interphases studied		% exact prediction
		Total	Y positive interphases	
11	M	115 000	51 (0.04%)	83
1		15 000	0 (0%)	
4	F	35 000	0 (0%)	
2		20 000	3 (0.02%)	

TABLE 5 Exactitude of sex prediction from Y chromosomes in metaphases

Mothers examined	Sex of child at birth	Metaphases studied		% exact prediction
		Total	Y positive metaphases	
10	M	12 949	15 (0.12%)	89
2		310	0 (0%)	
6	F	8163	0 (0%)	

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

Sex of child at birth	Interphases		Metaphases		No of mothers
	With Y body	Without Y body	With Y chromosome	Without Y chromosome	
F	+	-	+	-	0
	+	-	-	+	2
	-	+	+	-	0
	-	+	-	+	4
M	+	-	+	-	9
	+	-	-	+	2
	-	+	+	-	1
	-	+	-	+	0

percentage of correct sex predictions agrees with those found by other authors, both in metaphase counts (Walknowska *et al.*¹⁴ 83%; de Grouchy and Trébuchet,¹⁵ 67%; Schindler *et al.*¹⁶ 38%) and in interphase counts (Schröder and de la Chapelle,¹⁷ 71%; Grosset *et al.*¹⁸ 86%; Zilliacus *et al.*¹⁹ 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).

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.

FIG 1 Variation of frequency of positive Y bodies in interphase during pregnancy.

FIG 2 Variation of frequency of XY metaphases in the blood of pregnant women during pregnancy.

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 authors¹⁴⁻¹⁶ 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

FIG 3 Longitudinal variation per mother of Y body frequencies in interphase during pregnancy.

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.*,¹⁶ the 13th week by de Grouchy and Trébuchet,¹⁵ the 14th week by Grosset *et al.*¹⁸ and by Walknowska *et al.*,¹⁴ the 15th week by Zilliacus *et al.*,¹⁹ and the 16th week by Schröder and de la Chapelle.¹⁷

Moreover, although fetal lymphopoiesis is classically considered to start at the 16th week, Playfair *et al.*²² 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%.

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*¹⁶ on metaphases and by Schröder and de la Chapelle¹⁷ on interphases. Walknowska *et al*¹⁴ and de Grouchy and Trébuchet,¹⁵ 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*,¹⁶ 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 rapidly (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 asymmetry²³ or other polymorphisms.^{24 25}

The use of this method for routine prenatal diagnosis will therefore depend on the improvement of biochemical and immunological techniques. Turunen *et al*²⁶ distinguished fetal lymphocytes from maternal lymphocytes by a fluorescence-activated cell sorter. Also it is possible that some mitogens might selectively stimulate fetal cells.^{26 27}

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.

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