Prenatal diagnosis: techniques used to help in ruling out maternal cell contamination*

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SUMMARY The combined findings from a number of different analytical techniques increases confidence that the cells analysed in amniotic fluid cell cultures are fetal in origin. Three hundred and twenty four fluids were processed using *in situ* processing of cultured amniotic fluid cells, allowing for analysis of mitoses from multiple colonies derived from multiple culture dishes. Screening of the same samples for fluorescent Y-chromatin was of help in indicating the genotypic sex of the primary cells. This was found to be accurate in 96% of the fluids checked. In cases where an XX complement is found, Q-polymorphism comparisons can be made between mitoses from the amniotic fluid cells and maternal lymphocytes. Of 29 such studies, 19 showed pronounced differences in their polymorphism constitution.

The power of a programme in intrauterine diagnosis is directly dependent upon the accuracy of a laboratory diagnosis in the absence of the ability to examine the patient. One of the problems facing laboratories involved in prenatal diagnosis is the possibility that maternal cells may contaminate the amniotic fluid sample (Milunsky, 1973; Macintyre, 1971). This problem is illustrated by a case from our own series.

Case report

Only cells with a 46,XX complement were found in cultures from a fluid sample obtained from a patient whose indication for amniocentesis was that she was a carrier for Duchenne's muscular dystrophy. Amniocentesis was performed at 14 weeks' gestation, but 10 days later the fetus aborted spontaneously and was a phenotypic male. Unfortunately, no material was available to check the karyotype of the abortus.

Since the patient became pregnant with an intrauterine device in place, and since the effectiveness of such a device depends upon the production of a nidus of macrophages *in utero* (Sagiroglu and Sagiroglu, 1970; Sedlis and Reymak, 1970), it was

suggested that these migratory cells had invaded the amniotic fluid, and that these maternally derived cells were the ones which had been analysed.

Materials and methods

Three plastic centrifuge tubes (Falcon 2095) containing amniotic fluid were sent to the laboratory from each patient. The first tube had approximately 2 ml initial aspirate which might contain some blood. This sample was used to prepare slides for Y-chromatin screening by a modification of the technique described previously (Greensher *et al*, 1971). The remaining fluid, usually 10 to 20 ml, was used for culture preparation. 0.5 ml of the thoroughly mixed fluid sample was carefully deposited on a 22 mm² coverslip in each of six 35-mm plastic tissue culture dishes (Falcon No. 3001), the drop being confined to the coverslip. The remaining fluid was centrifuged at 25 g for 10 minutes, the supernatant removed, and the cells resuspended in 3.0 ml of medium (McCoy's 5A modified, supplemented with 5% fetal calf serum and 13% human cord serum). 0.5 ml cell suspension was similarly transferred to coverslips in 6 additional dishes. The 12 dishes were placed in a 37°C CO₂ incubator and 1 ml medium was added to each dish after 48 hours' incubation. The dishes were checked microscopically every 2 to 3 days at the same time that the medium was changed. The cultures should be washed with a number of medium changes if there is red cell contamination or large numbers of nonviable cells.

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When small colonies of actively dividing cells were seen, usually by 6 to 14 days, the medium was changed and on the following day the processing procedure was initiated. The cultures were incubated for two hours with colcemide (final concentration 0.0025 $\mu\text{g/ml}$). Keeping the dish level, the medium was then carefully removed from the edge of the dish with a Pasteur pipette. 2.0 ml cold 0.8% sodium citrate was added slowly down the side of the dish and allowed to stand at room temperature for 20 minutes. An equal volume of freshly prepared fixative (1:3 acetic acid:methanol) was slowly added to the hypotonic solution and after 2 minutes the total liquid volume was carefully removed. 2.0 ml fixative was added to the dish and removed after 20 minutes, and a second fixation was carried out for a similar interval. After removal of the fixative the coverslip was blown on directly three or four times and the dish inverted over the lid to allow excess fluid to drain off. The coverslip was now stained by a modification of the Seabright (1971) trypsin technique for G-banding. If a 46,XX karyotype was identified in the mitoses examined, coverslips were restrained with Atebrine by a modification of the technique described by Caspersson *et al* (1970), and then Q-polymorphism comparisons were made between these cells and similarly stained preparations from maternal lymphocyte cultures. The comparisons were made by noting the presence or absence of bright fluorescence in the centromere regions of chromosomes 3 and 4, and the short arm and satellite regions of chromosomes 13, 14, 15, 21, and 22. Because of the variables inherent in the technique, no attempt was made to identify and grade intermediate fluorescence levels as suggested by others (Hauge *et al*, 1975).

The current routine procedure in this laboratory relies heavily on the evaluation of multiple colonies from three or more coverslips to rule out maternal cell contamination, and Q-polymorphism comparisons were only carried out if less than three coverslips were available.

If no pronounced differences were found, a repeat specimen was obtained.

Results

Three hundred and twenty-four fluids were evaluated by the Y-chromatin screening method. The results are summarized in Table I. In 29

TABLE I
Y-CHROMATIN SCREENING OF 324 SAMPLES

	Chromosome Complement	
	XY	XX
Y-Chromatin positive	131	8
Y-Chromatin negative	4	152
Inadequate preparation	13	16

specimens (9.0%) insufficient satisfactory cells were found for quantitative analysis of the Y-chromatin status. Seven specimens (2.4%) were Y-chromatin positive despite a 46,XX karyotype. In 2 of these cases where quinacrine staining was performed on the chromosomes, one showed large fluorescent short arm of a chromosome No. 22 and the other a prominent fluorescent short arm of a chromosome No. 15. In the other 5 cases, a fluorescent karyotype was not prepared, and we can only infer that these too contained a highly fluorescent polymorphism. An eighth sample, also Y-chromatin positive, contained large numbers of leucocytes and yielded a 46,XX karyotype on initial culture, the coverslips showing many attached single cells but no colonies. Only a few mitoses were present probably derived from the contaminating maternal

FIG. Q-polymorphism comparison. Arrows designate polymorphic markers in the maternal cell complement which are not present in the amniotic fluid cell complement.

white cell population. Cultures prepared from a repeat specimen yielded colonies containing only mitoses with a 46,XY karyotype.

In the four samples that had a Y-chromatin negative result and a 46,XY karyotype, two showed the presence of a small Y-chromosome with an absent, or very small, fluorescent segment. In an epidemiological study we found approximately 1 in 1000 males to have a short Y chromosome (Goad *et al*, 1976). The difference in frequency is probably caused by the small sample in this present study. The reason for the results in the other two cases are unknown, but may have been the result of inadequate uptake of the stain.

The same 324 amniotic fluids were cultured and processed using the coverslip method, with the results shown in Table II.

TABLE II
STATISTICAL BREAKDOWN OF IN SITU PROCESSING
OF 324 CULTURES

	Range	Mean
Mitoses examined	2-40	12.3
Colonies	1-15	7.0
Petri dishes	1-10	4.2
Days in culture before processing	6-22	11.9

Note: The first 114 cultures in the series were analysed using 10 mitoses as a minimum requirement. This was increased to 15 mitoses for the latter 210.

In 29 recent cases where a 46,XX karyotype was found, 19 (64%) showed distinct differences between maternal lymphocyte and amniotic fluid cell Q-polymorphism constitutions.

Few cultures yielded results interpreted as pseudomosaicism, as only one colony of cells with an abnormal chromosome complement was present in each case. The details of these findings are published elsewhere (Peakman *et al*, 1975).

Discussion

Fluorescent Y body prescreening of amniotic fluid cell samples is useful, particularly when the sample is Y-chromatin positive and only 46,XX cells are cultured. In such a case it is necessary to consider a distinct fluorescent polymorphism or maternal cell contamination. Care needs to be taken in the evaluation of these preparations and only those of superior quality used for analysis. Because of these documented imperfections in the determination of the Y-chromatin status of uncultured amniotic fluid cells, it is imperative that this screening technique should not be depended upon for the determination of fetal sex.

Although previously described (Tjio and Puck, 1958; Abbo and Zellweger, 1970; Cox and Ray,

1971), yet not widely used, the *in situ* processing method has proved to be a useful technique in the analysis of amniotic fluid cell cultures. Conservation of mitoses means that fewer cells are needed than for the conventional trypsin harvest method, and thus a shorter culture period is required. This in turn can keep *in vitro* changes in chromosome constitution to a minimum. Only one slide has to be scanned for each dish processed, keeping the analysis time short. Its most important feature is that it allows for the analysis of individual colonies derived from single cells or small numbers of cells, assisting in the diagnosis of true and pseudomosaicism (Cox *et al*, 1974). The more colonies and dishes that are checked when a 46,XX complement is found, the greater the confidence that a fetal karyotype is being examined. This may be further confirmed if polymorphic differences in maternal and amniotic fluid cells can be identified. However, since this is an expensive and time-consuming procedure, it is used in this laboratory as a supplementary technique only when few colonies and coverslips are available for analysis.

The use of these methods should diminish the danger of misdiagnosis in genetic amniocentesis.

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