Culture of bloody amniotic fluid for chromosome analysis: an improved method

Contamination of amniotic fluid samples with maternal or fetal red blood cells occurs in approximately 5% of amniocenteses. Culturing these samples for fetal chromosome analysis is often difficult, particularly when blood clots are present, because many of the amniotic fluid cells may become trapped within the clot. The combined use of trypsin to break up the clot and of ficoll-based lymphocyte separation medium (LSM) was found to be satisfactory for separating red cells from amniotic fluid cells.

The procedure was as follows. Any clots present in the specimen of amniotic fluid were trypsinised at 37°C for 30 to 40 minutes using a trypsin-versene solution (0.025% lyophilised trypsin, 0.025% EDTA disodium salt in phosphate buffered saline).

Four types of culture were set up from each sample of amniotic fluid containing clots:

- (1) aliquots of untreated blood stained amniotic fluid;
- (2) aliquots of amniotic fluid treated with LSM to remove the majority of red cells (see below);
- (3) aliquots of trypsin cell suspension from the trypsinised clots; and
- (4) aliquots of the trypsin cell suspension which had also been subjected to LMS separation.

Standard methods of culture maintenance and harvesting for chromosome preparations were used.¹

Red cell separation technique

A total of 2 to 3 ml bloody amniotic fluid (or trypsin cell suspension) was layered carefully on to the surface of an equal volume of LSM (Flow Lab Cat No 16-920-46) in a conical centrifuge tube. The tube was centrifuged at 400 *g* for 10 minutes. Amniotic fluid cells remained in suspension, whereas the red cells sedimented to form a pellet. The supernatant layers, containing the amniotic fluid cells, were poured off, mixed with 3 ml Ham's F10 medium and centrifuged at 400 *g* for 10 minutes. The pellet of red cells was also resuspended in 3 ml of Ham's F10 medium and centrifuged at 400 *g* for 10 minutes.

The supernatants were poured off. The cell pellets were resuspended in Ham's F10 medium enriched with 15% fetal bovine serum and 15% AB human serum, seeded into Leighton tubes, gassed with 5% CO₂ in air, and incubated at 36.5°C.

Chromosome preparations were obtained from 35 of 36 amniotic fluid specimens which contained blood clots. In 25 cases (69%) the untreated samples failed to grow, but good results were obtained from at least one of cultures 2, 3, or 4.

In nine cases both the treated and untreated samples were successful. There was one exception where only the untreated sample gave results. The use of LSM rarely inhibited or retarded the cell growth, being less toxic to the amniotic fluid cells than the ammonium chloride technique which had been previously used for red cell elimination.²

The success rate of growth from samples containing blood clots, after the application of this method, has risen to 97%. This is 1% lower than that achieved for other amniotic fluid specimens in this laboratory.

The separation with LSM is also effective whenever the fluid is visibly contaminated with blood or has a 'cloudy-brownish' appearance because of the presence of cell debris.

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References

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- ² Lee CLY, Gregson NM, Walker S. Eliminating red blood cells from amniotic fluid samples. *Lancet* 1970;iii:316-7.

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