Combined use of alphafetoprotein and amniotic fluid cell morphology in early prenatal diagnosis of fetal abnormalities

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SUMMARY The combined use of alphafetoprotein (AFP) measurement and amniotic fluid cell morphology was assessed in 217 pregnancies with normal outcome (including 12 where an anterior placenta was traversed), and 52 where there was a fetal defect (25 cases of anencephaly, 21 of open spina bifida, 2 of exomphalos, 2 of urogenital atresia, and 2 of intrauterine death). In each case maternal serum and amniotic fluid AFP was measured. Total, viable, and rapidly adherent cells were counted and amniotic fluid cell morphology was examined. On the basis of this experience a scheme is suggested for more precise antenatal diagnosis of fetal abnormalities.

One of the most rapidly growing uses of diagnostic amniocentesis is for the mother whose indication is a raised serum alphafetoprotein (AFP) value. The Report of the United Kingdom Collaborative Study on Alphafetoprotein in Relation to Neural Tube Defects (1977) has documented the risk of fetal spina bifida and anencephaly in pregnancies with defined levels of maternal serum AFP. However, a high serum AFP may also indicate intrauterine death, threatened abortion, congenital nephrosis, exomphalos, duodenal or oesophageal atresias, or other fetal disorders (Seppala, 1977). Since a raised serum AFP is usually followed by diagnostic amniocentesis, it seems obvious that these amniotic fluids should be examined in ways most appropriate to the possible outcome of the pregnancy. In this paper we present evidence that careful examination of the number and properties of the amniotic fluid cells, together with amniotic fluid AFP assay, offers more precise information on fetal condition than the more conventional AFP assay alone.

Materials and methods

AMNIOTIC FLUIDS

Amniotic fluids were obtained at 15 to 24 weeks' gestation by transabdominal amniocentesis. Most were samples obtained because the mother had

2 successive serum AFP values above the 95th centile of the normal range. Some samples were from a second amniocentesis where the original indication had been maternal age, a previous affected child, or family history of genetic disease, and where the first amniocentesis specimen had a marginally raised amniotic fluid AFP value. In all the pregnancies involved, fetal karyotypes were reported within the normal time span for the laboratory.

AFP DETERMINATIONS

Maternal serum AFP concentrations were measured by a solid-phase radioimmunoassay developed by Dr Tony Bolton and described in detail elsewhere (Brock *et al.*, 1978). Amniotic fluid AFP was determined by rocket immunoelectrophoresis (Brock and Sutcliffe, 1972). The upper limit of normal for each week of gestation has already been defined (Brock *et al.*, 1975).

DETERMINATION OF AMNIOTIC FLUID AND BLOOD CELL NUMBERS

The number of total and viable amniotic fluid cells was determined in a sample of whole amniotic fluid. 0.1 ml amniotic fluid was incubated with 0.02 ml Trypan blue at 37°C for 5 minutes. The mixture was placed in Neubauer counting chambers, the total and viable amniotic fluid cell numbers recorded, and the number of blood cells counted. Cell smears were made of all samples where blood cells were present. These were

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stained by the Shepherd or Kleihauer methods with appropriate adult and fetal blood cell controls, and the proportions of maternal and fetal blood in the amniotic fluid were thus determined.

CELL CULTURE FOR 20-HOUR CELLULAR ADHERENCE

After estimation of the number of viable cells, an aliquot of amniotic fluid was taken, so that approximately 2000 viable cells were suspended over each large coverslip. (If viable counts were very low, the number of cells for planting was reduced to try and ensure that no more than 2 ml amniotic fluid was used for this procedure.) All media used for rapidly adherent cell growth had to be supplemented with at least 20% serum. This serum was always pretested to ensure that it supported successful growth of amniotic fluid cells. Fetal calf serum which supported good and rapid cell growth in culture was satisfactory, and in our hands always proved superior to newborn calf serum. Human AB serum was excellent in its ability to enhance abnormal cellular adherence; the disadvantage was that large batches of serum had to be tested for cytotoxic properties, and suitable sera pooled and stored frozen in aliquots in order to achieve consistent results. We have found Ham's F10 tissue culture medium to be readily available commercially and extremely good as a growth medium for amniotic fluid cells, but any rich medium which will support good growth of primary amniotic fluid cells may be used. An important factor is the pH maintained in chambers of settling cells which should not at any stage exceed 7.6 during the culture period. This is most easily achieved using HEPES buffer. Other buffers such as bicarbonate-CO₂ or MOPS can be used, but the equilibrating and final pH must be carefully checked. Failure to keep the pH between 7.0 and 7.2 affects cellular adherence.

PLATING FOR CELLULAR ADHERENCE

0.5 ml amniotic fluid with cells was added to 4.5 ml of Ham's F10 medium buffered with HEPES, supplemented with 25% fetal calf serum, 50 units/ml penicillin, and 50 µg/ml streptomycin. This mixture was divided equally between the sterile 50 mm plastic petri dishes, one containing three 9 × 35 mm glass coverslips and one containing a single 22 × 40 mm glass coverslip. The coverslips were prepared by soaking in methanol, flame drying from the methanol, and then autoclaving between layers of Whatman no. 1 filter paper (non-autoclaved coverslips yielded fewer adherent cells). The petri dishes were incubated at 37°C in a hydrated atmosphere. After 20 hours' incubation, the coverslips were removed, washed twice with phosphate-buffered saline, fixed in methanol, and stained with May-Grünwald Giemsa.

RAPIDLY ADHERENT CELL TYPES

The rapidly adherent cells were classified by their morphology as described in Gosden and Brock (1977) and Gosden *et al.* (1977). The major groupings are:

(1) *Neural cells*. These comprise long cells (both bipolar and fibroblastic types), cells with fine filamentous pseudopodia, large vacuolated cells, and multinucleated giant vacuolated cells.

(2) *Placental cells*. These differ from neural cells in lacking cytoplasmic granulations and inclusions. Their most characteristic feature is an eccentrically placed nucleus appearing as a 'cap' at one end of the cell. Occasionally multinucleates are found, but these are small groups which lack vacuolations and cytoplasmic bridges.

(3) *Peritoneal cells*. These are of 2 main groups, the peritoneal macrophages which have cytoplasmic vacuolation, and cells with eccentrically placed nuclei.

(4) *Epithelioid cells*. These are large pale-staining squamous cells which usually have a small nucleus.

Results

CELL NUMBERS

Counts of the number of total and viable amniotic fluid cells were made in 217 fluids obtained between 15 and 24 weeks' gestation from pregnancies where the outcome was a normal fetus. The total cell counts per ml amniotic fluid at each gestation are shown in Fig. 1 with values from the abnormal fetuses (25 cases of anencephaly, 21 cases of spina bifida, 2 cases of exomphalos, 2 cases of intrauterine death, and 2 cases of urogenital atresia) shown as open circles. It can be seen that, in general, cell numbers increase as gestation progresses, though there is considerable scatter. In part this variation may be the result of the 3 different methods of estimating gestation; clinical assessment, from the date of LMP, and by ultrasound measurement of biparietal diameter.

The relation of viable cell numbers to gestation is shown in Fig. 2, with the cell counts per ml amniotic fluid from the abnormal fetuses seen as open circles. The viable cell counts show rather less variation than total cell counts. Where maternal blood cells were present in large numbers there were problems of the maternal blood clots trapping viable cells and thus reducing cell numbers. In samples with no maternal blood, only the fluids from the 4 cases of urogenital atresia and intrauterine death had viable cell numbers of less than 600 cells/ml amniotic fluid. An interesting case is that of fetus A25/7 where the gestation was estimated to be 16 weeks from the ultrasound measurement of biparietal diameter, but was 19 weeks by dates (Table 1). The viable cell number of 21 000 cells/ml is clearly very high for a gestation of 16 weeks (Fig. 2). There were rapidly adherent neural cells in

Fig. 1 Total cell numbers per ml amniotic fluid at different weeks of gestation.

Fig. 2 Viable cell numbers per ml amniotic fluid at different weeks of gestation.

Table 1* Alphafetoprotein and cellular values of amniotic fluid samples from pregnancies with abnormal fetuses

Case no.	Serum AFP (ng/ml)†	Amniotic fluid AFP (µg/ml)‡	Viable cell nos. (per ml)	% of cells viable	Cellular adherence (%) (viable cells)§	Gestation** (weeks)	Fetus
A42/5	230 (18)	50 {17}	7800	10	12	19	SB
A52/5	147 (19)	58 {21}	13 300	29	11	20	SB
A58/5	470 (17)	323 {24}	10 600	10	91	20	ASB
A65/5	423 (23)	69 {10}	16 700	23	21	22	A
A18/6	238 (16)	144 {24}	12 700	40	29	22	A
A53/6	195 (17)	117 {17}	8700	27	31	20	ASB
A60/6	190 (19)	33 {21}	25 400	50	9	19	SB
A65/6	>500 (18)	89 {24}	6000	40	89	18	A
A70/6	345 (17)	64 {17}	12 000	36	10	20	ASB
A95/6	175 (16)	61 {31}	12 000	51	13	16	ISB
A97/6	250 (18)	117 {21}	4700	35	97	19	ASB
A98/6	>400 (21)	85 {14}	16 000	38	91	21	A
A103/6	245 (18)	143 {24}	8000	32	100	18	A
A105/6	160 (20)	20 {14}	8700	31	28	22	SB
A106/6	340 (17)	156 {24}	660	5	53	18	A
A107/6	240 (18)	38 {14}	12 000	27	9	21	SB
A108/6	300 (18)	163 {21}	11 300	31	52	19	A
A2/7	155 (16)	49 {24}	8600	20	14	18	SB(AP)
A3/7	220 (16)	134 {31}	6600	76	44	16	A
A4/7	410 (18)	147 {21}	9300	56	25	19	A
A6/7	235 (18)	150 {28}	700	13	33	17	ASB
A8/7	105 (15)	146 {28}	700	8	25	17	A
A18/7	250 (18)	147 {21}	8700	42	100	19	ASB
A24/7	190 (16)	46 {24}	10 600	50	13	18	SB
A25/7	98 (16)	22 {31}	21 300	43	6	16	SB
A26/7	360 (17)	160 {31}	4000	54	45	17	ASB
A57/7	380 (17)	60 {28}	4700	28	12	17	SB
A63/7	390 (18)	118 {24}	8600	46	100	18	A
A64/7	380 (17)	69 {28}	12 700	38	18	17	SB
A66/7	295 (18)	81 {24}	22 600	51	12	18	SB
A67/7	210 (18)	30 {10}	16 000	16	18	22	SB
A83/7	110 (19)	26 {17}	14 700	18	24	20	SB
A87/7	370 (18)	184 {17}	14 700	31	34	18	A
A103/7	283 (19)	125 {13}	4700	13	35	19	ASB
A104/7	253 (17)	108 {21}	7300	27	53	17	ASB + O
A104/6	400 (19)	170 {21}	15 300	36	12	19	O
A84/7	>500 (19)	147 {13}	22 700	51	10	19	O
A12/7	135 (17)	4 {28}	300	5	5	19	UA
A15/7	125 (19)	30 {21}	18 000	40	2	19	N (AP)

*Table 1 contains only those pregnancies where serum AFP values were available.

†Gestation at which serum value estimated in brackets.

‡Upper limit of normal in brackets.

§Normal range of cellular adherence in 20 hours is 0-1 to 6%.

**Gestation at amniotic fluid AFP estimation and cell counting.

SB, spina bifida; A, anencephaly; ISB, iniencephaly with spina bifida; O, exomphalos; AP, anterior placenta; UA, urogenital atresia.

the amniotic fluid, and if the amniotic fluid AFP value is taken at 19 weeks (the more probable gestation indicated by cell numbers), it is clearly abnormal, whereas at 16 weeks it is normal. The fetus had spina bifida.

CELLULAR ADHERENCE AS A PROPORTION OF VIABLE OR TOTAL CELLS

Cellular adherence is expressed in 2 ways. Fig. 3 shows the number of cells adherent to glass in 20 hours expressed as a percentage of viable cells. The upper limit for amniotic fluids from normal fetuses is 6%. The proportion of viable cells adhering is, in general, greater for anencephalic fetuses than for those with spina bifida (Table 1, Fig. 3). Amniotic fluid cells from 2 pregnancies where the fetus had exomphalos showed that approximately 10% of the viable cells

planted were adherent to glass. When expressed as a proportion of the viable cells, the cellular adherence values for amniotic fluids from fetuses with neural tube defects or exomphalos give no false positive or false negative values.

Fig. 4 shows the adherent cells as a percentage of the total cell number in amniotic fluids. The upper limit of normal is 3%, but it can be seen that some values for fetuses with neural tube defects are below this. Furthermore, the information on the size and type of the lesion, which is quite clear from the viable adherent cell percentage, is to a very large extent lost when total cell numbers are used. Both anencephalic and spina bifida cell values fall beneath, or at the upper limit of, normal for total adherent cell percentages. Attempts to measure cellular adherence without cell counts give many false positive and false negative values; cell counts improve the resolution of the technique, but

Fig. 3 Cellular adherence at 20 hours as a percentage of viable cells. The upper limit of normal (given by the maximum number of adherent epithelioid cells from amniotic fluids from normal fetuses, where the placenta was not traversed during amniocentesis) is defined as 6%.

Fig. 4 Cellular adherence at 20 hours as a percentage of total cells. The upper limit of normal (given by the maximum number of adherent epithelioid cells from amniotic fluids from normal fetuses, where the placenta was not traversed during amniocentesis) is defined as 6%.

only viable cell counts are satisfactory for diagnostic results.

RAPIDLY ADHERENT CELL TYPES AND PROPORTIONS

In pregnancies where the fetus is normal, the adherent cell type (in proportions less than 6% of the viable cells suspended over the cover slip) is the epithelioid cell. The only exception to this is where there is a large anterior placenta which has been directly traversed during amniocentesis. In these cases, some small non-vacuolated cells with eccentric nuclei adhere to the coverslips, usually in proportions less than 5% of the viable cells. In certain cases of repeat amniocentesis, this proportion may rise to 40%. These cells are termed placental cells (Table 2).

Where the fetus is abnormal, different cell types from the amniotic fluid are adherent to glass in 20 hours. In the case of anencephaly and open spina bifida, long cells, cells with multiple processes, vacuolated cells, and multinucleated giant vacuolated cells are present, and these are collectively termed neural cells (Table 2). Where the fetus has exomphalos, peritoneal cells pass into the amniotic fluid. The proportion of abnormal adherent cells usually gives an indication of the size of the lesion. Fetuses which have anencephaly and rachischisis have up to 100% of rapidly adherent viable cells (Fig. 3), and certainly the majority of anencephalic fetuses give cellular values which exceed 30%. Amniotic fluids from fetuses with spina bifida have only about 9 to 25% of adherent viable cells, and the maximum value of 50% has been found in only 1 fetus of the 21 with spina bifida examined.

An interesting difference is shown in the amniotic fluid cells from the fetus with iniencephaly and spina bifida (A95/6) (Fig. 5 and 6) when compared with the cells from the fetus with exomphalos (A84/7) (Fig. 7). The amniotic fluid from the iniencephalic obtained at 16 weeks' gestation had 13% adherent viable cells while that from the exomphalos had 10% (Table 1). Though there was a similarity in the number of adherent cells, the cell types were very different (Table 2). The iniencephalic fluid had neural cells present, while the fluid from the fetus with exomphalos had only peritoneal cells. A further difference is seen in the AFP values in the amniotic fluid. The iniencephalic with spina bifida had an AFP value of 61 $\mu\text{g/ml}$ (that is, in the normal spina bifida range) (Table 1), while that of the exomphalos was very high at 147 $\mu\text{g/ml}$. This is as high or higher than anencephalics with complete rachischisis. Fig. 8 shows the relation between amniotic fluid AFP values and the cellular adherence for viable cells. The trend is for the anencephalics to have values exceeding those of the spina bifida pregnancies in both measures. In contrast,

Table 2 Rapidly adhering cells as an aid to the differential diagnosis of fetal abnormality

Condition	Neural cells	Peritoneal cells	Placental cells	Epithelioid cells
Cellular adherence greater than 6%				
Anencephaly (25 cases)	100%	—	—	*
Spina bifida (21 cases)	100%	—	—	*
Exomphalos (2 cases)	—	100%	—	*
Placental tap (2 cases)	—	—	100%	*
Cellular adherence less than 6%				
Normal fetus (205 cases)	—	—	—	100%
Anterior placenta (normal AFP) (10 cases)	—	—	20-90%	10-80%
Urogenital atresia (2 cases)	—	—	—	100%
Intrauterine death (2 cases)	—	—	—	100%

*Occasional epithelioid cells seen in these samples.

Fig. 5 Fetus with iniencephaly and spina bifida (A95/6) at 17 weeks' gestation (lateral view).

Fig. 6 Iniencephalic fetus (A95/6) showing lesion.

the pregnancies with fetal exomphalos show clearly the high AFP values, but only moderately raised cellular adherence values.

Discussion

It has been known for some time that amniotic fluids from pregnancies where the fetus has a gross open neural tube defect contain high proportions of cells which adhere rapidly to glass surfaces (Sutherland *et al.*, 1973; Nelson *et al.*, 1974). Originally, these cells

were termed macrophages by virtue of the ability of some of them to phagocytose sensitised sheep red cells (Sutherland *et al.*, 1975). We have previously noted that they consist of a variety of different morphologies and have renamed them functionally as rapidly-adhering (RA) cells (Gosden and Brock, 1977). They appear to originate from the fetal brain and spinal cord (Gosden *et al.*, 1977), and though mainly evident in cases of fetal anencephaly, may also be seen in small open spina bifida lesions (Brock and Gosden, 1977a). A significant finding has been that RA cells of quite

Fig. 7 Fetus with exomphalos (gastroschis) (A84/7) after termination at 19 weeks' gestation.

distinctive morphology appear in the amniotic fluid when the fetus has exomphalos or when the placenta has been traumatised (Gosden and Brock, 1977). This has led us to suggest that antenatal diagnosis of congenital malformations should be based not on AFP assay alone, but also on detailed examination of the amniotic fluid cells (Brock and Gosden, 1977b). In this paper we present the evidence for this assertion.

Counts of total and viable cells increase with advancing gestation (Fig. 1 and 2), as has been noted by previous workers (Nelson, 1973). The distribution of viable cells shows less scatter than that of total cells. None of the abnormal pregnancies had unusually high cell counts, though in case A25/7 the viable cell count was helpful in assessing the dis-

Fig. 8 Relation between percentage cellular adherence for viable cells and amniotic fluid AFP concentrations in $\mu\text{g/ml}$. Symbols as in Fig. 3 and 4.

crepancy between gestation based on LMP and that based on ultrasonar estimate of biparietal diameter. Though the latter is usually regarded as the more reliable, there is no rigorous evidence in published reports to substantiate this belief, and we argue that viable cell counting should not be ignored. Of the blood-free fluids, 4 had unusually low cell counts. These corresponded to 2 cases of intrauterine death (not detected at amniocentesis) and 2 cases of urogenital atresia. But the principal value of cell counting is that it gives a quantitative measure of red blood cells in blood-stained fluids. When this is combined with Kleihauer staining it provides an accurate estimate of fetal blood contamination. This is of cardinal importance in assessing the significance of raised amniotic fluid AFP levels in blood-stained fluids (Brock, 1977).

In many amniotic fluids where the fetus has anencephaly, very large numbers of RA cells can be observed, thus rendering quantitative estimates redundant. However, the diagnosis of fetal anencephaly by amniotic fluid AFP assay is seldom a problem, and in our experience of 45 cases values were always more than 10 SD above the mean value for the week of gestation (Brock, 1977). Where quantitative RA cell counting is necessary is in the diagnosis of spina bifida and in the differential diagnosis of neural tube defects and other fetal abnormalities, such as exomphalos. Here we have shown that RA cell proportions must be based on viable cell counting rather than total cell counting (Fig. 3 and 4). Total cell counting, though marginally easier, obscures information on the type of lesion and leads to significant numbers of normal proportions for severe congenital lesions (Fig. 4). In

general, the proportion of viable cells which are rapidly adherent correlates well with amniotic fluid AFP, both increasing in cases of large, open lesions. It is noteworthy that the 2 cases of exomphalos stand out by having high AFP values and comparatively low RA cell proportions (Fig. 8).

Though cell counting is important, the real value of amniotic fluid cell morphology lies in the variety of cellular types which may be encountered. In this paper we have classified RA cells into 4 main groups: neural cells, peritoneal cells, placental cells, and epithelioid cells (Table 2). These have been described in detail and their origins discussed elsewhere (Gosden *et al.*, 1977). Epithelioid cells are, of course, seen in all amniotic fluids, but their adherence rate is low and if the fetus has a neural tube defect or exomphalos, or if the placenta is grossly traumatised, they may not be detectable on a coverslip removed after 20 hours in culture (Table 2). Individual cell types may overlap between groups (for example, vacuolated cells are found in both neural and peritoneal groups), but in general the groupings are distinct and we have had little difficulty in the differential diagnosis of neural tube defects, exomphalos, and normal fetuses with traumatised placentae on the basis of cell morphology. It is also worth noting that in 2 cases of intrauterine death, where the amniotic fluid AFP values were greater than 10 SD above the mean, the few RA cells encountered were epithelioid in nature.

In Table 3, a scheme for the differential diagnosis of fetal abnormalities is presented. It uses serum and amniotic fluid AFP, total, viable, and RA cell counts, and the examination of the morphology of those cells adherent after 20 hours in culture. It is based on a comparatively limited experience (217 normal pregnancies and 52 fetal abnormalities), and must therefore be seen as tentative. It is our hope that it will serve as an amendable model for those concerned with definitive antenatal diagnosis of fetal defects.

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References

- Brock, D. J. H. (1977). Review. Prenatal diagnosis of neural tube defects. *European Journal of Clinical Investigation*, **7**, 465-472.
- Brock, D. J. H., Barron, L., Manson, J., and McCrae, W. M. (1978). Serum alphafetoprotein in cystic fibrosis of the pancreas. *Clinica Chimica Acta*, **82**, 101-103.
- Brock, D. J. H., and Gosden, C. (1977a). Early antenatal diagnosis of small open spina bifida lesions. *British Medical Journal*, **2**, 934.
- Brock, D. J. H., and Gosden, C. (1977b). Are second trimester amniotic fluids being properly examined? *Lancet*, **2**, 1168-1169.
- Brock, D. J. H., Scrimgeour, J. B., and Nelson, M. M. (1975). Amniotic fluid alphafetoprotein measurements in the early prenatal diagnosis of central nervous system disorders. *Clinical Genetics*, **7**, 163-169.
- Brock, D. J. H., and Sutcliffe, R. G. (1972). Alpha-fetoprotein in the antenatal diagnosis of anencephaly and spina bifida. *Lancet*, **2**, 197-199.
- Gosden, C. M., and Brock, D. J. H. (1977). Morphology of rapidly adhering amniotic fluid cells as an aid to the diagnosis of neural tube defects. *Lancet*, **1**, 919-922.
- Gosden, C. M., Brock, D. J. H., and Eason, P. (1977). The origin of the rapidly adhering cells found in amniotic fluids from foetuses with neural tube defects. *Clinical Genetics*, **12**, 193-201.
- Nelson, M. M. (1973). Amniotic fluid cell culture and chromosome studies. In *Antenatal Diagnosis of Genetic Disease*, pp. 69-81. Ed. by A. E. H. Emery. Churchill Livingstone, Edinburgh.
- Nelson, M. M., Ruttiman, M. T., and Brock, D. J. H. (1974). Predictive value of amniotic fluid macrophages in gross C.N.S. defects. *Lancet*, **1**, 504.
- Report of United Kingdom Collaborative Study on Alphafetoprotein in Relation to Neural Tube Defects (1977). *Lancet*, **1**, 1323-1333.

Table 3 Differential diagnosis of fetal abnormality using AFP values and cellular data

Serum AFP	Amniotic fluid AFP	Cell no. (Total)	Cell no. (Viable)	Cellular adherence (Viable cells)	Cell morphology	Fetus
Raised	Normal (<3 SD)	Normal	Normal	<6%	Epithelioid	Normal
Raised	Raised (>3 SD)	Normal	Normal	>6%	Neural	Spina bifida
Raised	Raised (>3 SD)	Normal	Normal	1 to 40%	Placental	Anterior placenta (normal fetus)
Raised	Normal (<3 SD)	Low	Very low	<6%	Epithelioid	Urogenital atresia
High	High (>10 SD)	Normal	Normal	>20%	Neural	ASB
High	High (>10 SD)	Normal	Normal	6 to 20%	Peritoneal	Exomphalos
High	High (>10 SD)	Normal	Low	<6%	Epithelioid	Intrauterine death

- Seppala, M. (1977). Review. Immunologic detection of alphafeto-protein as a marker of fetal pathology. *Clinical Obstetrics and Gynecology*, **20**, 737-757.
- Sutherland, G. R., Brock, D. J. H., and Scrimgeour, J. B. (1973). Amniotic fluid macrophages and anencephaly. (Letter) *Lancet*, **2**, 1098-1099.
- Sutherland, G. R., Brock, D. J. H., and Scrimgeour, J. B. (1975). Amniotic fluid macrophages and the antenatal diagnosis of anencephaly and spina bifida. *Journal of Medical Genetics*, **12**, 135-137.
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