CULTURE METHOD FOR AMNIOTIC FLUID CELLS

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The effect of the period of pregnancy and the concentration of the cell suspension to be cultured on success in the culture of amniotic fluid cells was studied on 29 samples. The optimal time for taking amniotic fluid for quick and successful culture was found to be the 17th week of pregnancy. The need to allow for the concentration of the cell suspension before addition of the cells to the culture medium, depending on the period of pregnancy, was demonstrated.

KEY WORDS: amniotic fluid cell culture; amniocentesis; cytogenetic analysis.

The main task in the antenatal diagnosis of inherited diseases by means of amniotic fluid cell culture is rapid establishment of the diagnosis. To solve this problem it is not sufficient to obtain successful cell cultures, but the culture time must also be reduced to the minimum. The optimal culture time of amniotic fluid cells is known to be 2-3 weeks on the average [1]. To determine the antenatal diagnosis, the investigator thus requires on average 3-4 weeks. The urgency of the solution to this problem of rapid cell culture in order to establish the diagnosis will be obvious. Usually the optimal time for taking amniotic fluid is considered to be the second trimester of pregnancy [1, 3]. However, the problem of the more precise time during pregnancy for the performance of amniocentesis in order to obtain the most rapid possible culture of amniotic cells has not yet been settled.

This paper gives the results of a study of the effect of the conditions of culture and the periods of pregnancy on growth of amniotic cells in vitro with the aim of reducing their culture time.

EXPERIMENTAL METHOD

The investigation was carried out on amniotic fluid cells (29 specimens) obtained at termination of pregnancy (14-23 weeks) on medical grounds. The volume of amniotic fluid in the different specimens varied from 100 to 200 ml, so that in each case several scores of variants of the cultures could be obtained. In this way the effect of various factors on growth of amniotic cells could be tested.

Glass 40-ml flasks and Carrel's jars (diameter 50 mm) were used to transport the amniotic fluid from the maternity home, and they were sent to the laboratory in the horizontal position (the "position of culture"). The amniotic fluid cells were then sedimented by centrifugation at 100 rpm for 10 min and transferred to other vessels for culture in different concentrations. Since the most viable amniotic fluid cells attach themselves to the substrate within a relatively short time (1-2 h), medium also was poured into the vessels in which the fluid was transported and they were used for culture. Cells were cultured in Carrel's jars, glass flasks, and also in penicillin flasks (diameter 19-21 mm) with a coverslip on the bottom for cytogenetic analysis. The nutrient medium used consisted of 80% medium No. 199 (Institute of Poliomyelitis and Virus Encephalitis, Academy of Medical Sciences of the USSR) and 20% embryonic calf serum (Microbiological Associates), with the addition of glutamic acid (up to a concentration of 0.03%) and antibiotics (100 units penicillin, 50 units streptomycin, and 100 units monomycin to 1 ml culture medium). It is important to note that seedings of the cells were successful also when human group AB serum obtained from several donors was used instead of embryonic calf serum, but the efficiency of culture was 65% compared with 91%.

Before addition of the cells to the culture the total number of cells in the specimen was counted by means of a Goryaev's counter and the number of viable cells was determined by staining them with a 0.4% aqueous solution of tryphan blue. Cells were added to the culture medium only if the specimen contained not less than 8-10% of viable cells. The first change of medium in the culture vessels took place on the 6th or 7th day of

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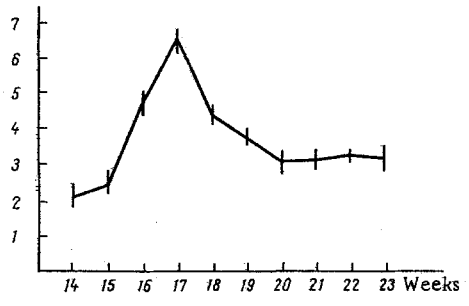

Fig. 1

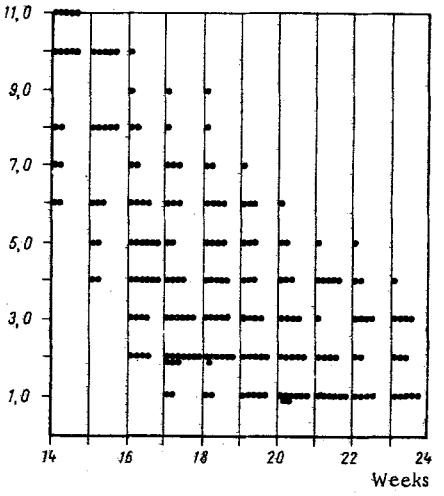

Fig. 2

Fig. 1. Number of colonies in cultures of amniotic cells on 7th day of culture from different samples. Abscissa, stage of pregnancy (in weeks); ordinate, number of colonies in culture.

Fig. 2. Success of culture of amniotic cells depending on concentration of cell suspension cultured and stage of pregnancy. Abscissa, stage of pregnancy (in weeks); ordinate, number of cells ($\times 10^5$) in 1 ml. Each point indicates presence of growth on 7th day in culture.

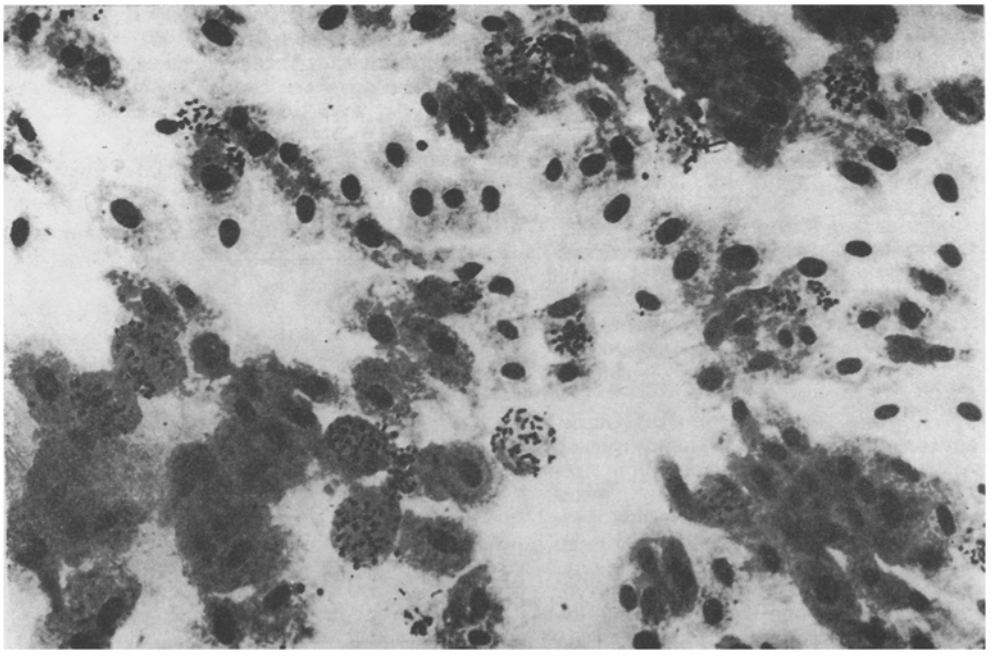

Fig. 3. Fragment of preparation obtained for cytogenetic analysis on 9th day of culture. Azure-eosin, 100×.

culture. Later the medium was changed every 2-3 days until sufficient cells were available for cytogenetic analysis. Culture was regarded as successful if at least two or three growing colonies consisting of not less than 20 cells were found on the 7th or 8th day.

Chromosome preparations of the amniotic cells were obtained by culturing the cells in penicillin flasks on the surface of a coverslip. Colchicine was added to the flasks 18 h after the change of medium in a concentration of 0.5 μg/ml for 5 h. Hypotonic treatment was carried out with distilled water mixed with bovine

serum in the ratio of 4:1 with the addition of hyaluronidase (2.5 units/ml). The cells were fixed 22-24 h after the previous change of medium with Carnoy's fixative (twice, for 30 and 15 min respectively).

EXPERIMENTAL RESULTS

Adhesion of amniotic cells and their growth in culture are known to depend on the ratio between the numbers of living and nonviable cells in the fluid, which in turn depends on the stage of pregnancy [1, 2, 3]. When colonies were counted in different cultures on the 7th-8th day in culture, they were most numerous if the amniotic fluid was taken at the 17th week of pregnancy (Fig. 1). The difference in the number of colonies between the 16th and 17th weeks, and also between the 17th and 18th weeks of pregnancy was significant ($P < 0.001$).

Since the ratio between the number of living and dead cells changes during pregnancy, by changing the total concentration of cells before their addition to the culture in samples taken at different stages of pregnancy, it is possible to obtain cultures in a short time, namely 8-10 days (Fig. 2). If the sample of amniotic fluid is taken at the 16th-17th week of pregnancy, the cells must be cultured in an initial concentration of $2.0 \cdot 10^5$-$4.0 \cdot 10^5$ cells/ml (Fig. 2).

If amniotic fluid is taken at the 17th week of pregnancy, and if cells are added to the culture in a concentration of $2.0 \cdot 10^5$ cells/ml, it is thus possible to obtain a sufficient number of colonies of dividing cells on the 8th-9th day of culture for complete cytogenetic analysis (Fig. 3).

If the amniotic fluid is taken at other times, by making allowance for the cell concentration before commencing culture, rapid and successful culture of amniotic cells can also be achieved (Fig. 2).

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