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INFLUENCE OF DIFFERENT TISSUE CULTURE MEDIA AND SERUM CONCENTRATIONS ON DEVELOPMENT AND KARYOTYPE OF FL-CELLS IN VITRO

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Abstract: Four combinations of tissue culture media, different concentrations normal calf serum and their influence on the development and karyotype of a human amniotic cell line FL have been studied. Optimal combination for growth and development of the cell line is HD + 5 % NCS.

Karyotype evolution of the cell line FL shows a tendency of passing from triploidy (71 after FOGH & LUND) to normal diploid karyotype.

Keywords: FL-cells, tissue culture media, serum, cell proliferation, modal class chromosomes.

Cell heterogeneity of most cell lines is subject to use of different cultivation techniques, different tissue culture media, sera, antibiotics and other experimental agents.

According to MAMAEVA (1996) essence karyotype characteristics of continuous cell lines are: decrease of chromosome number cells variability, presence of frequently expressed modal class of cells, minimum karyotype heterogeneity among cells and a balanced karyotype.

Well known is that in the process of cell lines prolonged cultivation different characteristics could change, including the karyotype ones. Different culture media and sera are factors leading to such changes.

Influence of two culture media and different serum concentrations on cell proliferation and modal chromosome number of the cell line FL is present in this paper.

MATERIAL AND METHODS

Cell culture.

Cell line FL (normal human amnion cells), isolated by FOGH & LUND in 1956

(ATCC, 1993, CCL 62) was used, cultivated in medium LY (0.5 % lactalbumin hydrolisate, 0.1 % yeast extract and 0.4 % dextrose in Earle's BSS). The FL-cells were kindly placed at our disposal by Dr. Trajancheva from the Laboratory of Virology (HEI, Plovdiv), where they have been cultivated in a tissue culture MEM with HANKS (30 %), 10 % calf serum, 5 % lactalbumin and antibiotics (penicillin, streptomycin, canamycin).

In our laboratory the cells were adapted and cultivated in tissue culture HAM'S F12 + DMEM (1:1) - SERVA, 10 % normal calf serum (NCS), 100 IU/ml penicillin and 100 mg/ml streptomycin. They were incubated in CO_2-incubator HERAEUS at 37° C, and high humidity. Tripsinization and subcultivating were done according to INVITTOX (Protocol 3b/1990), adapted for FL-cells.

Experiment.

The experimental model includes four variants:

1) HAM'S F12 + DMEM (HD) + 10 % NCS;
2) HAM'S F12 + DMEM (HD) + 5 % NCS;
3) HAM'S F12 + DMEM (HD) + 1 % NCS;
4) DMEM (D) + 10 % NCS.

After 3 consecutive subcultivations cells were seeded on glass lamellae in 5 cm petri dishes for more 3 consecutive passages. For each variant time of cultivation was 120 hours, while on every 24 hours a fixation was performed. Cells were fixed with methanol for 7 minutes and stained with Giemsa for 40 minutes. Initial cell density for all variants and subcultivations was 4.5×10^4 cells/ml.

Mitotic index was calculated by the formula:

$$MI\ (‰) = \frac{\text{number of dividing cells} \cdot 1000}{\text{total cell number}}$$

where by each calculation the total cell number was 1000.

Doubling time was calculated after SCHAEFFER (1978):

$$T = \frac{t.lg2}{lg(N/N_0)},$$

where T is doubling time, t - part of time corresponding to the culture logarithmic growth phase, N_0 - number of cells in the beginning of the given period of time, and N - number of cells at the end of the same period.

For the chromosome analysis to the culture medium for each variant was added colchicine (0.2 μg/ml) for 3 hours. Chromosome plates were prepared after CONKIE et al. (1989).

Results were statistically processed and are presented as mean value of 3 independent experiments, each of them triple repeated.

RESULTS

The influence of different culture media and serum concentrations on cell proliferation and karyotype of the cell line FL was estimated by mitotic index (MI), doubling time (between 24 and 48 hours) as well as modal chromosome number.

The higher mitotic index (MI) values are observe by HD + 10% NCS and D + 10 % NCS. Intermediate values are these by HD + 5 % NCS and low by HD + 1 % NCS.

Up to the 72-hour in all four variants MI increases (fig. 1). After this hour by D + 10 % NCS and HD + 1 % NCS, MI decreases, and by the 120-hour they reach 116.00 ‰ and 68.67 ‰. By HD + 10 % NCS and HD + 5 % NCS even after the 72-hour dividing cells increase and MI-values by the 96-hour are 196.67 ‰ and 146.33 ‰ respectively, after which (on the 120-hour) they decrease, reaching 139.00 ‰ and 135.33 ‰ (fig. 1). At the same figure is seen that the optimum profile has the MI-curved line for medium HD + 5 % NCS, because it increases steady up to the 96-hour, and afterwards a decrease is very easy in comparison with the other variants.

Doubling time is calculated for a 24-48 hour interval, corresponding to the logarithmic cell culture growth. For HD + 10 % NCS it is 33.22 hours, for HD + 5% NCS - 27.47 hours, for HD + 1 % NCS - 27.73 hours and for D + 10% NCS - 73.02 hours (fig. 2). Graph shows, the shortest doubling time has the variant HD + 5 % NCS again.

For the karyotype determination 100 metaphase plates of each variant were analyzed. The result is that the modal chromosome number for cells, cultivation in HD + 10 % NCS is 49 (from 40 to 53) - fig. 3. For cells, cultivating in HD + 5 % NCS it is 44 (from 37 to 51, rarely 56 and 79); for HD + 1 % NCS - 47 (from 38 to 55, rarely 86), and for D + 10 % NCS - 47 (from 40 to 56) - fig. 3.

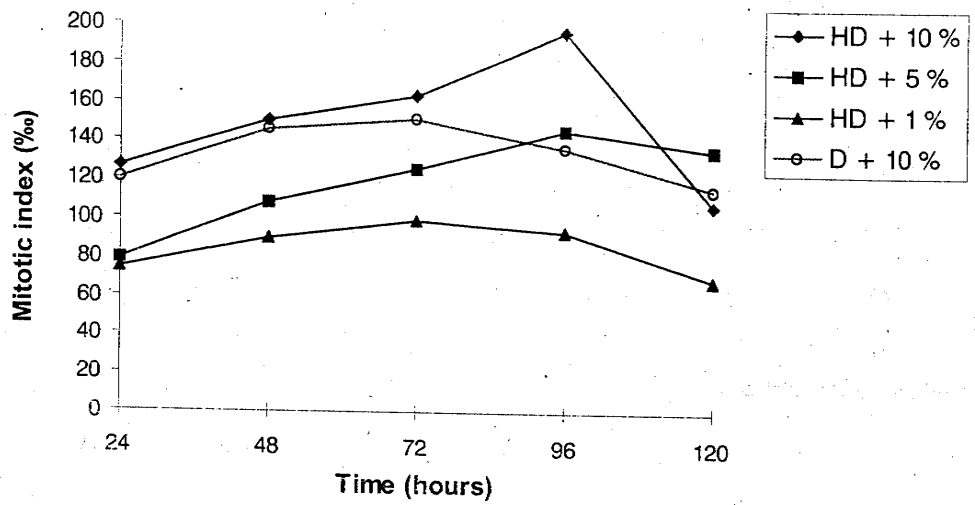
Karyotype of the initial cell line FL, taken from HEI, Plovdiv was determined. The modal chromosome number varies - 47, 51 and 52 (fig. 4) in comparison with the four experiments.

DISCUSSION

Results from mitotic index and doubling time studies show that both media for cultivating of FL-cells (HD and D) ensure good conditions for the culture development, with higher MI-values and growth rate for HD. There is a proportional dependency between serum concentration in the medium and the rate of cell proliferation, where HD + 5 % NCS is an optimal medium combination for cultivating of FL-cells.

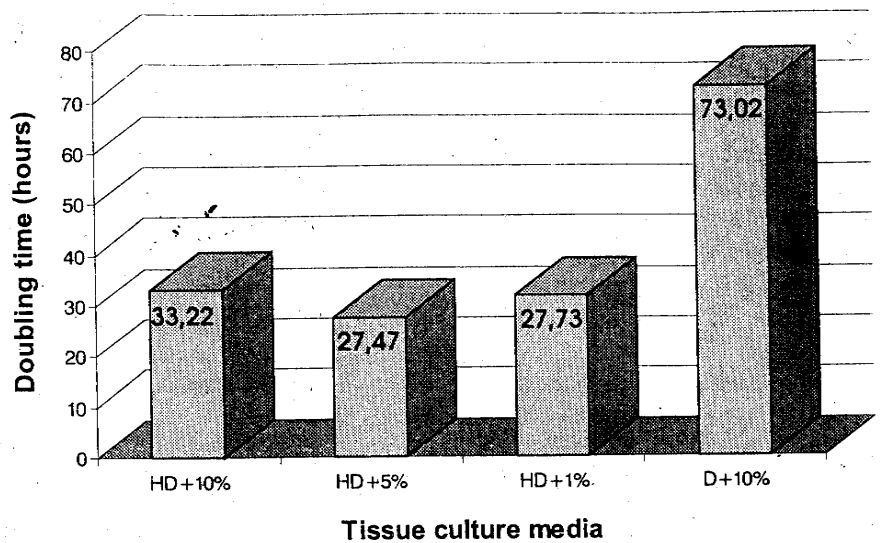
Under prolonged cultivation for most cell lines a chromosomal polymorphism is characteristic, where in each chromosome could occur different structural transformations. By the different cultivation conditions, most probably survive separate clones of FL, with an exact modal number and type of chromosomes, which provide the cell line development in these very conditions.

Comparing the chromosomes modal number from all four experimental variants and the initial cell line FL (HEI) with the established by MIKHAILOVA et al. (1978) modal number (60) for FL-cells and the karyogramme of the originally isolated (1956) cell line FL by FOGH & LUND, we observe that the karyotype of FL-cells, cultivated in different conditions is different as well (fig. 5). Karyotype evolution of the cell line FL shows a tendency from triploidy (71 by FOGH & LUND) to normal diploid karyotype.



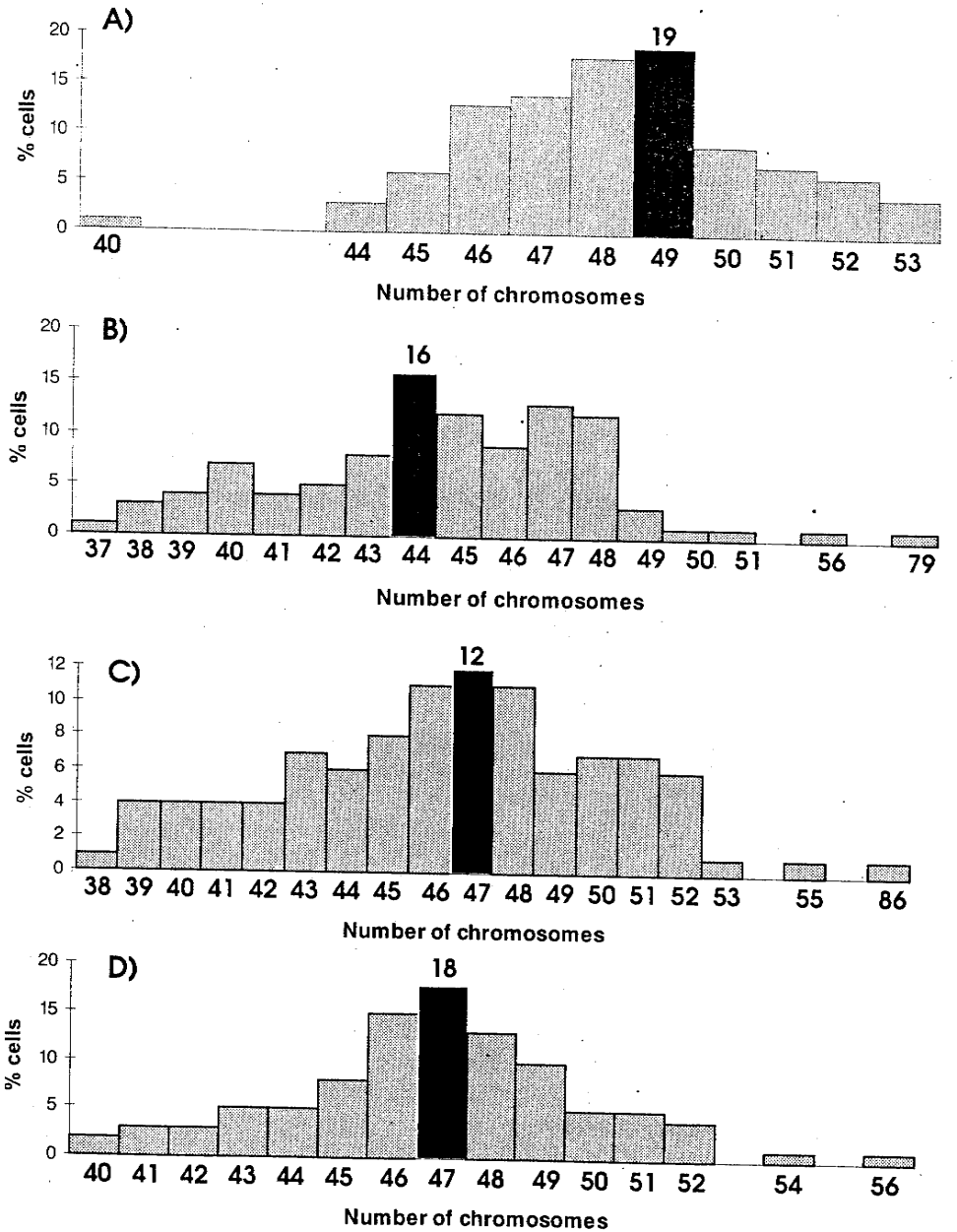
Фигура 1. Динамика на митотичния индекс (MI) за 24, 48, 72, 96 и 120 часа инкубационно време на FL-клетки, култивирани в хранителни среди HD + 10 % NCS, HD + 5 % NCS, HD + 1 % NCS и D + 10 % NCS.

Figure 1. Mitotic index (MI) dynamics for 24, 48, 72, 96 and 120 hours incubation time for FL-cells, cultivated in culture media HD + 10 % NCS, HD + 5 % NCS, HD + 1 % NCS and D + 10 % NCS.



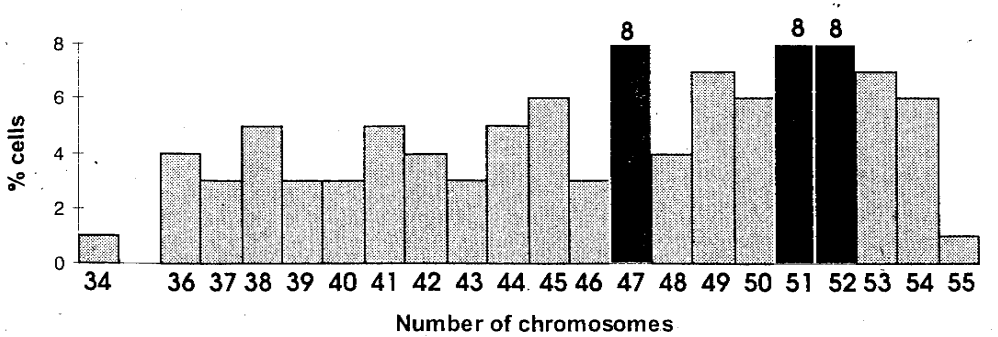
Фигура 2. Време на удвояване на клетъчната численост за 24 часа (в интервала 24-48 часа) на клетки от клетъчна линия FL, култивирани в хранителни среди HD + 10 % NCS, HD + 5 % NCS, HD + 1 % NCS и D + 10 % NCS.

Figure 2. Cell number doubling time for 24 hours (between 24-48 hours) of cell line FL, cultivated in culture media HD + 10 % NCS, HD + 5 % NCS, HD + 1 % NCS and D + 10 % NCS.



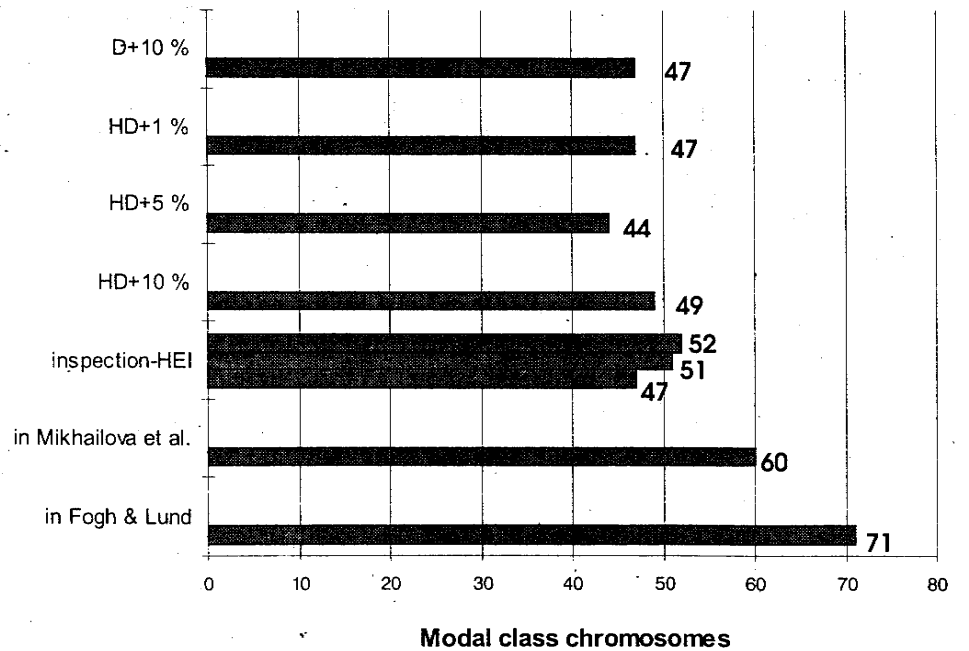
Фигура 3. Разпределение на броя хромозоми във FL-клетки, култивирани в:
A) HD + 10 % NCS;
B) HD + 5 % NCS;
C) HD + 1 % NCS;
D) D + 10 % NCS;

Figure 3. Chromosomes number distribution in FL-cells, cultivated in:
A) HD + 10 % NCS;
B) HD + 5 % NCS;
C) HD + 1 % NCS;
D) D + 10 % NCS;



Фигура 4. Разпределение на броя хромозоми в клетки от клетъчна линия FL(ХЕИ, Пловдив), култивирани в хранителна среда MEM (HANKS) + 10 % NCS + 5 % лакталбумин.

Figure 4. Chromosomes number distribution in cell line FL (HEI, Plovdiv), cultivated in culture medium MEM (HANKS) + 10 % NCS + 5 % lactalbumine.



Фигура 5. Модален брой хромозоми на клетъчна линия FL, култивирана при различни условия.

Figure 5. Chromosomes modal number of cell line Fl, cultivated in different conditions.

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ВЛИЯНИЕ НА РАЗЛИЧНИ ХРАНИТЕЛНИ СРЕДИ И СЕРУМНИ КОНЦЕНТРАЦИИ ВЪРХУ РАЗВИТИЕТО И ХРОМОЗОМНИЯ НАБОР НА FL-КЛЕТКИ IN VITRO

Балик М. ДЖАМБАЗОВ, Никола К. ПОПОВ, Георги С. КЪРНЕВ

(Резюме)

Хетерогенността на повечето клетъчни линии, използвани в експерименталната биология, е обусловена от различните техники на култивиране, различни среди, серуми, антибиотици и други агенти.

В настоящата работа е изследвано влиянието на две хранителни среди (HAM'S F12 и DMEM), както и различни серумни концентрации (нормален телешки серум, NCS) върху клетъчната пролиферация и модалния брой хромозоми на клетъчна линия FL. Експериментирано е с четири комбинации (среда + серум). Влиянието е оценявано по митотичен индекс, време за удвояване (между 24 и 48 час) и модален брой хромозоми.

Оптимална среда за култивиране на FL-клетките е HD + 5 % NCS. Кариотипната еволюция на клетъчна линия FL показва една тенденция на преминаване от триплойден (71 по FOGH & LUND) към нормален диплоиден кариотип.