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Effects of seed, serum and media composition on growth and proliferation of BHK cells in suspension culture in a stirred bioreactor

S.S. Parizi[1], M. Ardjmand[2], H. Mahravani[3*], M. Azimi[3]

1-Department of Chemical Engineering , Science and Research branch, Islamic Azad University, Tehran, Iran.
2- Department of Chemical Engineering, South Tehran branch, Islamic Azad University, Tehran, Iran
3- Foot and mouth disease reference laboratory, Razi vaccine & serum research institute, Agricultural Research Education and Extension Organization (AREEO), Karaj, Iran

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The present study focus on the effects of the seed, serum and media composition including protein, glucose, amino acids, glutamine and vitamins on the BHK[1] cells growth and proliferation. All experiments have been done in suspension culture in a 1-liter bioreactor equipped with U-type blades mixer and air Ventilation exchange. This bioreactor are widely used for process development and laboratory studies of production cell line. Considering multiplication ratio, doubling time and specific growth rate, effect of each studied kinetic parameter on BHK cells was also investigated. Higher multiplication ratio and specific growth rate, lower doubling time and make the cell culture growth in well condition. The results, show that the best amount of cell seed is $3*10^5$- $4*10^5$ for starting culture. The amount of 15% calf serum obtain the highest number of cells in the end of culture process. Glucose, protein and glutamine have the greatest impact on the growth and proliferation of BHK cells, respectively. The purpose of present research is to effect the different factors in suspension of BHK cells line for high density production.

Keywords: mammalian cell culture, supplements, seeds, BHK cells, bioreactor, suspension

INTRODUCTION

Cell and tissue culture devised in the early twentieth century is an artificial process for growth, proliferation and maintenance of cells for study and research on the behavior of various animal cells [1-3].

Cell culture is mainly applied in biological sciences such as virus propagation, vaccine production, diagnosis, hormones production, physiological , biological , pharmaceutical studies, basic , applied researches including cell biology, physiology, pharmacology and toxicology, recombinant protein production , gene therapy, cancer research , drug development, antibodies, interferon, erythropoietin, coagulation factors , safety testing and many other aspects [1,4-8].

The use of cell culture has grown significantly and is constantly developing and progressing [8].

Although the first culture of mammalian cells to study cell physiology was carried out in the early twentieth century, it has taken 50 years for the cell culture to reach the industrial scale [9].

The first commercial product of cell culture was poliomyelitis vaccine produced from monkey kidney cells culture [10-11].

The cells used in cell culture are divided into two sub-categories. Primary cells as the first category are taken directly from living tissues and cell lines as the second category are infinitely cultivated under the laboratory conditions in any amount. some properties of cell line are population doubling time, ability to grow in suspension, saturation density (yield per monolayer dishes) [3,12-13].

Cell lines are taken from immortal cells and are used in research experiments. Cells are grown under epithelial or fibroblastic conditions and are cultivated as suspension [3].

Working with cell lines is much easier than primary cells. Some of the cell lines are MDCK[2]-HeLa[3]-BHK-CHO[4]-HEK-293[5]-NS0[6] used in virus laboratory and protein production [3, 14].

BHK cells are derived from syrian baby hamster kidney (Mesocricetus auratus) [15].

BHK cells are inherently anchorage dependent cells but also they are applied as suspension, too [16-18].

To whom all correspondence should be sent:
E-mail: h.mahravani@rvsri.ac.ir

[1] Baby hamster kidney
[2] Madin Darby canine kidney
[3] Henrietta Lacks
[4] Chinese hamster ovary
[5] Human Embryonic Kidney 293
[6] Nonsecreting null

BHK cells are mainly used in animal products, particularly for foot and mouth disease vaccine (FMDV) and rabies vaccine production. They are also used to produce recombinant proteins such as factor IIIV and ELISA antigen for Japanese encephalitis virus (JE) and to extract DNA from Pseudo rabies virus.[4,5,19-21]

Adherent BHK cells are cultivated on the glass or plastic surfaces such as flasks, rollers and multi-layered dishes.[22]

Pseudo suspension and suspension culture in this type of cells is highly considered because monolayer culture for large scale quantities is not possible [20, 23-24].

Development of suspension culture systems allows increasing the number of cells, their biomass concentration and the volumetric efficiency in most cases, facilitating incubation conditions in scale-up and improving growth medium composition Suspension culture is preferred to various applications since the homogeneous control of the process and its scale change is much more convenient [25-26].

The ability of some cells to adapt suspension culture and the use of polymeric materials in order to reducing shear stresses on the cells make the suspension culture easier.[26]

Keeping cell culture under the sterile conditions and achieving the highest possible number of cells are two important principles in production using cell culture, affected by many factors.[26-27]

The most essential and important point in choosing the culture medium is the cell type used in the cell culture process.[1]

The culture medium is one of the most important factors in cell and tissue culture. A suitable medium must contain all nutrients required for cellular growth, proliferation, and metabolism. An overall culture medium is a mix of essential and non-essential amino acids, vitamins, mineral salts, glucose and serum. In medium without serum, keeping cells alive is difficult.[28-31]

The culture medium offers such services as biosynthetic precursors for anabolism, catabolic substrates for metabolism energy, vitamins and trace elements having the primary catalytic role, mineral ions mass (electrolyte) having both a catalytic role and a physiological role in controlling pH and osmolality in the acceptable range.[32]

Serum is the source of hormones, growth factors.The animal serum is added to the base culture medium. Enriching medium with the different animal serums is essential for cells growth and proliferation (Mitogenic effect). The used serums are taken from fetal and adult animals. Serum is generally a complex matter containing large amounts of high and low molecular weight compounds, promoters and growth inhibitors compounds, albumin, amino acids, proteins, vitamins, especially fat-soluble like A, D, E, K, carbohydrates, lipids, minerals and trace elements. The most important roles of serum in cell culture consist of the provision of hormones stimulating the cell growth and proliferation, movement of mineral proteins and trace elements and lipids, adherent and promoting factors, maintaining pH and resistance to toxic substances [32-34]

Fetal calf (bovine) serums and calf serum are the main sources of serums [34].

Amino acids are the basic building blocks of proteins and essential amino acids should be added into the culture media [35].

Glutamine is one of the most important amino acids and the main source of nitrogen.[36-37]

Carbohydrates are as sugars and in most cases, the culture media contain glucose which is the main source of carbon and energy for cell growth but some media include galactose, maltose and fructose.[36-38]

Albumin, transferrin, and fibronectin are the most proteins present in the culture media.[38]

Vitamins affect cell growth, proliferation and biochemistry.[29,39,40]

Among the mammalian cells, BHK is widely used in the vaccine productions, biotechnology, diagnosis and treatment. However, due to low production and low cell density, in general, yield in mammalian cell culture is low and this is undesirable for production. Progress in the process of mammalian cell culture is possible in two ways: The first way is improving the production process and the second one is improving the culture medium. Glucose and glutamine are two main materials used by mammalian cells in the process of growth and proliferation.[36]

In this study, the effect of cell seeds amount, the amount of calf serum and some components of culture medium used in the suspension culture conditions in bioreactor on growth and proliferation of BHK cells was investigated. Various kinetic parameters such as multiplication ratio, particularly the doubling time and specific growth rate are also examined.

MATERIAL AND METHOD:

2-1-Cell line, serum, culture media:

BHK cell line obtained from (foot and mouth disease national references laboratory, Razi vaccine and serum research institute, Karaj, Iran) was

cultured in MEM[7] supplemented with a set of proteins including lactalbumin, New Zealand casein, yeast extract and peptone were added into this medium, 2 % sodium bicarbonate, and 5% PEG[8] treated calf serum prepared by Razi institute with antibiotics penicillin 100 IU/ml and streptomycin 100 IU/ml (Gibco BRL, Rockville, USA).

2.2. Growth assay

BHK cells were initially cultivated in T-flasks and then scaled up to bottle at 36.5°C in a humidified atmosphere with 5% CO_2. The initial number of the cells was 2×10^5 cells/cm^2. In order to adapt the cells in suspension culture, a bottle system was used. The experiments were carried out in a glassware bottle (GLS 80®, Schott Duran, Germany) with a 1 L volume. pH of the medium (7.1±0.1) was controlled by CO_2. Culture temperature was 36.5°C±0.1. The stirring speed was maintained at 120-130 rpm. . Agitation was provided by magnetic u type impeller and equipped with air Ventilation exchange have spin filter (pore size0.02 _m) fixed on the axis. The bottle was inoculated with 5×10^5cells ml^{-1}. Then the cells under the suspension and mentioned conditions passed three passages to enter the testing phase. The experiments were carried out in triplicate. Samples were taken daily for cell count, cell morphology. All transfer activities were performed inside a laminar flow cabinet.

2.3. Cell counting

Cells were counted using a Fuchs-Rosenthal hem cytometer and by using the NucleoCounter™ NC-100 (automated cell counter systems, Chemo Tec , Cydevang , Denmark) according to the manufactures protocol. Viable and non-viable cells were determined by Trypan blue exclusion method. Viable cells are impermeable to Trypan blue and therefore the cells are transparent while non-viable cells are blue-dyed. The specific growth rate μ (h^{-1}) was estimated by the following equation:

$$\mu=\ln X_n - \ln X_{n-1})/(t_n - t_{n-1})$$

where X represents the viable cell density per ml, t represents the time-points of sampling expressed in hours and the subscripts n and $n–1$ stand for two successive sampling points.

To calculate the multiplication ratio (M.R) following formula was used:

$$M.R=\frac{Cf}{Ci}$$

Where final number of cells at the harvest time (C_F) by initial cell seed (C_i):

For calculate the doubling time (D.T) following formula was used:

$$D.T=\frac{\log 2*\text{culture time}}{\log \text{number of cell @ start} - \log \text{number of cell @ harvest}}$$

It is necessary to multiply time difference at the beginning of cell culture and harvest, which was always 48 hours in this experiment, in log 2 and divide by “log seed - log harvest”.

2.4. Morphological studies:

The morphology of the cells was regularly detected under inverted light microscope (nikon, Japan) throughout the work.

RESULT AND DISCUSSION

3.1. Effects of seeds on growth and proliferation of BHK cells

The experiments were performed using the culture medium MEM and 5% PEG treated calf serum. As shown in Table 1, effect of initial seeds on growth and proliferation of BHK cells has an ascending trend at the beginning of experiments and thereafter it has a descending trend. The highest number of cells equals to 1.44 *10^5 and 1.29*10^5 corresponding to 4*10^5 and 5*10^5 seeds, respectively (Table 1).

This can be attributed to the cellular competition for obtaining the nutrients material in the culture medium. During this competition, some cells are degraded. On the other hand, the initial seed influences to pH of media. Meanwhile, the materials are produced from cells growth and proliferation themselves stimulate growth.

As expected, the number of cells increased at the beginning of the culture process and their number increased after 48 hours and at the end of culture process. But after seeding 4*10^5 cells, this trend changed and decreased. The highest multiplication rate was for culture of experiment number 3.

As seen in Fig 1,the increasing trend of multiplication rate is observed until Experiment 3. The highest multiplication rate is in Experiment 3 in which the initial seed amount is 300,000 cells per ml, and equals to 3.7. After that, the descending trend is observed in the Fig1. This trend can be observed until the end of the experiments.

In all seven primary experiments related to 100,000 to 700,000 seed cells per ml, the doubling time is less than 100 hours. The highest doubling time, 722.5 hours, is for 900,000 seed cells per ml.(Fig.2)

[7] Minimum essential media

[8] Polyethylene glycol

According to Fig 3, the highest specific growth rate is in Experiment number 3 and 4. After Experiment 4 until the end of the experiments related to the effect of seed on BHK cells growth and proliferation in suspension culture, a descending trend is observed for the specific growth rate. The lowest specific growth rate that can be calculated is in Experiment 9 with 900000 seeds per ml and is equal to 0.001. The specific growth rate cannot be calculated for Experiment 10 but it show in Fig with 0.

The results of this study are in accordance with the experimental conditions proposed by other scientists. The results of this study are consistent with the working conditions used by Hassan, Cruz et al. and Tamis et al.[41-43]

Table 1: The effect of seeds on the final cell number in suspension culture and with the culture medium MEM and 5% PEG treated calf serum.

Experiment No.	Number of cells at the Start per ml (Trypan blue)*10000±30000	Number of cells at the Start per ml (Nucleo count) *10000	Number of cells at the harvest per ml (Trypan blue)*10000	Number of cells at the harvest per ml (Nucleocount)*10000
1	10±1	11±1	29±3	28±3
2	21±2	20±2	68±3	69±3
3	31±2	31±2	111±3	111±3
4	40±2	40±2	144±4	145±3
5	52±2	51±2	129±4	129±4
6	61±2	61±2	112±3	109±3
7	72±2	72±2	103±3	102±3
8	80±3	82±2	99±3	100±3
9	92±3	90±3	97±3	97±3
10	101±3	101±3	86±3	84±3

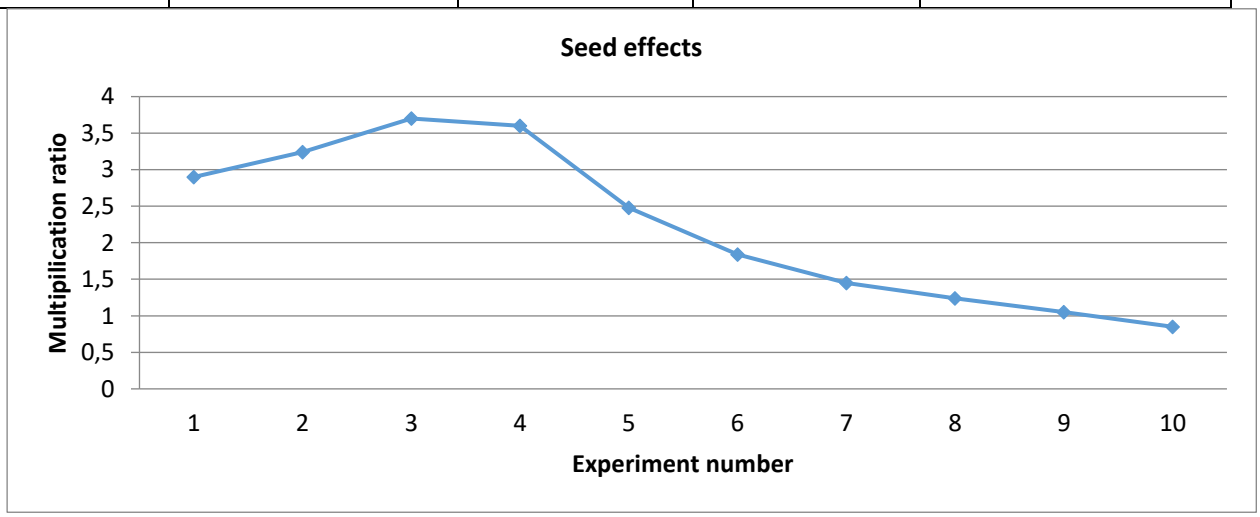

Fig 1,Multipilication ratio in different initial seed.(According to table 1)

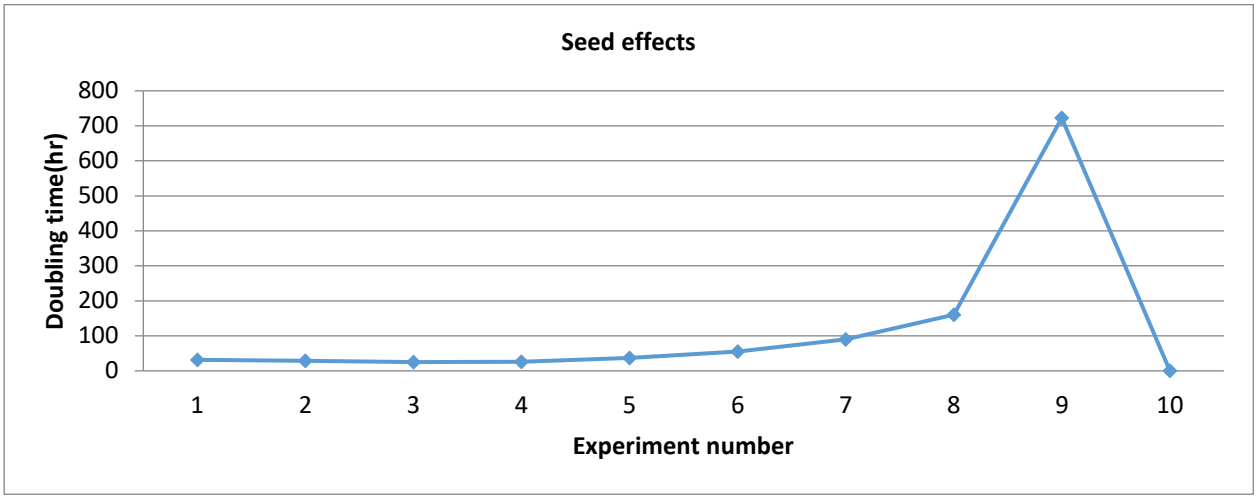

Fig2,Doubling time in different initial seed. (According to table 1)

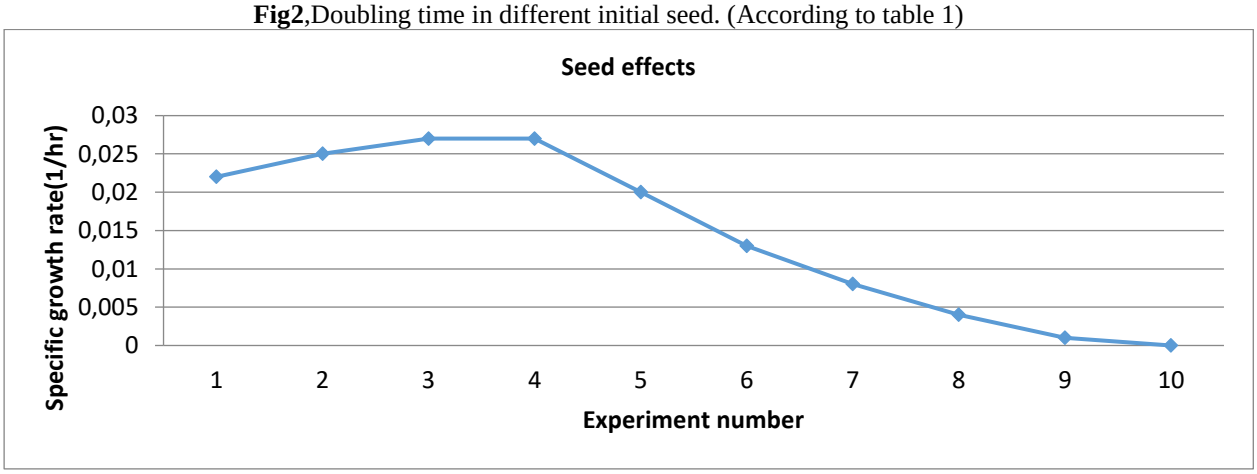

Fig3,Specific growth rate in different initial seed. (According to table 1)

3 .2. Effects of serum on growth and proliferation of BHK cells

The experiments were performed using the culture medium MEM and initial seed of $3.5*10^5$. The effect of serum amount on growth and proliferation of BHK cells is represented in Table 2. In general, the presence of serum improves the cell culture. Without serum, it can be said that the growth and proliferation of cells has not been efficient. The results of this study show that the absence of serum in the culture medium decreases the cell population at the end of culture process. The highest number of cells at the end of culture process which is obtained by 15% serum equals to $1.56 *10^5$. The absence of serum also decreased the number of cells at the end of culture process compared to the beginning of culture process. These cases prove the importance of the presence of serum and its impact on the BHK cells.

As seen in Fig 4, the highest multiplication rate is in Experiment 7 in which 15% serum has been used. The multiplication rate with this serum amount is 4.46. Also, in Experiment 1 with serum amount 0, the multiplication rate is less than 1. In general, the presence of serum improves the cell culture. Without serum, it can be said that the growth and proliferation of cells has not been efficient. The results of this study show that the absence of serum in the culture medium decreases the cell population at the end of culture process. Because serum is as a major source of growth hormones, trace elements such as zinc, copper and selenium, lipid and growth factors. Selenium is a powerful detoxifier and thereby is more effective in removing oxygen free radicals

As it can be seen in Fig 5, the doubling time in Experiment 1 is 0 because number of cells at the end of the culture is less than number of cells at the beginning of the culture.

The highest specific growth rate is in Experiments 7, 6 and 5, respectively, which their serum levels are 15% and 12.5% and 10%. The highest specific growth rate is equal to 0.031 (Fig. 6).

Serum is a great source of albumin, vitamins, minerals, amino acids, protein and carbohydrate. Vitamins are precursors of many co-factors. Many vitamins, especially vitamin group B (such as riboflavin, thiamin and biotin), are integral components in the process of cell growth and

proliferation. Serum also contains fat -soluble vitamins like A, D, E, K. tricarboxylic acid is one of the trace elements serum also absorb the toxic ammonium. Serum added into the culture media contains the main nutrients for cells, various proteins such as albumin which carries fat, hormones, fibronectin creating more and better connectivity of cell to substrate and transferrin that does not carry other molecules with itself. The serum also contains protease inhibitors protecting cells against pyrolysis and provides minerals such as $Na^{+,}$ K^{+}, Zn^{2+} and Fe^{2+}. Furthermore, on average, it increases the viscosity and thus, protects the cells against the shearing stress caused by the motion of the blades and other factors. The serum also acts as a buffer [33-35, 44-48].

All of the above are reasons causing cells not to well grow and proliferate well in the absence or low amount of serum in the culture medium. In this case, cell death occurs. But the addition of serum improves the situation and cell counting. This result is also consistent with the results of Rahman et al [21].

After using 15% serum, the ascending trend in cell number was stopped and the number of cells decreased with an increase in serum amount.

With the addition of serum and increase in the number of cells, the cells grow better and this trend is ascending and continues up to 15% (v / v) serum. But then this trend becomes descending. In addition to growth factors, serum contains growth limiting factors, too [46-48]. More than 15% serum Excessive increase in serum amount in the culture medium increases these factors and this has adverse effects on the growth and proliferation of BHK cells.

Reducing the amount of serum has a negative impact on the growth, proliferation, survival and morphology of cells in suspension culture conditions. This result is also consistent with the results of Durrani et al. and Hesham et al. experiments [1, 49].

In spite of the importance of serum and the need for it in the culture medium, there are also some problems with serum using. The first problem with taking serum from animals, especially animal fetus, is that this is morally incorrect and also costly. The major consumed serum around the world has been reported to be about 500,000 liters which is obtained through fetuses. In some cases, serum contains infectious substances such as bovine viral diarrhea, mycoplasma and chlamydia. In fact, between 20-50% of commercial FBS serum contains virus. Each of these factors is detrimental to the growth and proliferation of cells. Another problem by using serum in cell culture is its quality differences in different batches. Summer serums differ from winter serums due to the different livestock feed. Therefore, this point should be noted in using serums. Also, serums are problematic in situations such as purification of proteins and vaccines [45-49].

Despite the greatest impact of using 15% serum on the growth and proliferation of cells, however, due to the reasons such as reducing the suffering of animals, lower cost, lower risk of infection, and need for serum in the culture medium used in this study, we recommend the use of 5-10% serum. Our results showed that the highest rate of cell growth and proliferation is when 15% serum is used but good results can be achieved with lower serum amounts, too. These results are consistent with those of Paranjabh et al. and Harrison's researches showing that adding 2-10% serum has the greatest impact and higher percentages of serum do not cause significant differences.[45] If other proteins such as peptone and lactalbumin are applied, then serum is used 2% less, but certainly in protein-free culture medium, 5-10% serum is used.

Table2.The effect of serum amount on the final cell number in suspension culture and with the culture medium MEM and initial seed of $3.5*10^5$.

Experiment No.	Serum amount(v/v)	Number of cells at harvest per ml (Trypan blue) *10000	Number of cells at harvest per ml (Nucleo count) *10000
1	0	28±3	29±3
2	2.5	71±3	72±3
3	5	121±4	123±4
4	7.5	127±4	126±4
5	10	139±4	140±4
6	12.5	148±4	147±4
7	15	156±5	156±5
8	17.5	126±4	124±3
9	20	99±3	99±3

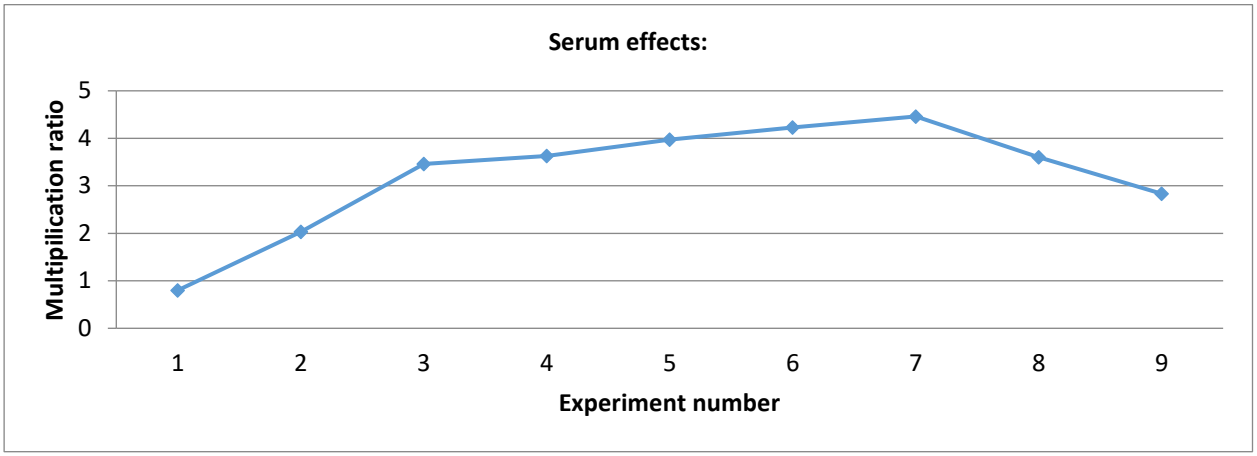

Fig4,Multipilication ratio in different percent serum.(According to table2)

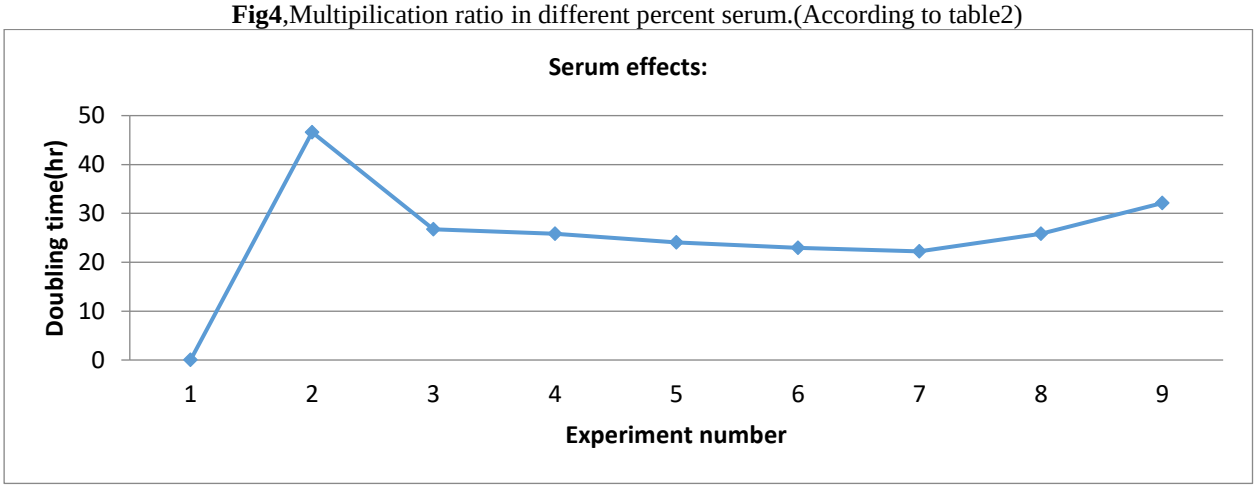

Fig5,Doubling time in different percent serum. (According to table2)

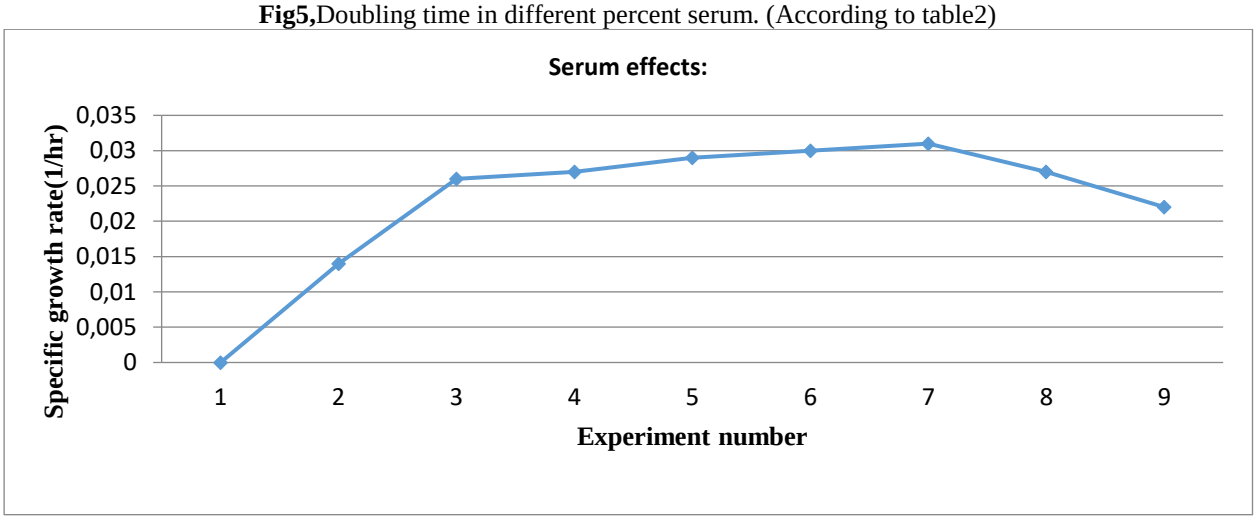

Fig6,Specific growth rate in different percent serum. (According to table2)

3.3. Effects of culture composition on growth and proliferation of BHK cells

In order to perform experiments to study the effect of supplements on the BHK cells, cells were cultivated in MEM without glucose, MEM without protein, MEM without glutamine, MEM without essential amino acids, MEM without vitamin and MEM with non-essential amino acids and complete media(MEM) with initial seed of 3.5* 10^5 and 5% calf serum.

Overall, most researches performed on BHK cells focus on the chemical parameters and nutrients such as glucose and glutamine in culture medium [29, 42, 50].

As time passed, it was established that each cell requires its own specific nutrients [51].

The culture medium is one of the most important factors in cell and tissue culture. A suitable culture medium must contain all nutrients

needed for cellular growth, proliferation and metabolism [28-29].

The culture medium contains inorganic salts, carbohydrates, amino acids, vitamins, fatty acids, lipids, proteins, peptides, serum and other additives such as glutamine, sodium pyruvate, and non-essential amino acidsCells need a culture medium with pH = 7.2-7.4 to grow and proliferate.[30-31,44]

The results of the conducted experiments on BHK cells in different mediums, each lacking a set of main components of the culture medium, are very interesting.

The greatest number of cells at the end of the culture process is in the culture medium containing all components and the results of this experiment are as expected.

The highest specific growth rate is for cells cultivated in the MEM. After that, the highest number of cultivated and proliferated cells is in the medium without vitamin. Protein-and glucose-free mediums with multiplication rates 0.63 and 0.54, respectively, had the lowest multiplication ratio during experiments.

BHK cells had the lowest growth in the medium without glucose. The reason is that carbohydrates and in particular glucose are the main sources of carbon and energy. However, some mediums also contain galactose, maltose or fructose.In the absence of this material, cells don't have the energy source that is their primary need for life and this is why they not only fail to grow and proliferate, but also their population decreases compared to the initial seeds. The amount of glucose present in serum is also not enough for cells to survive, grow and proliferate using it. As the absence of glucose in the medium causes some problems, the presence of glucose in the culture medium produces lactate. This substance is harmful and toxic for cells and inhibits their growth and proliferation. Because of its important role in cell growth, glucose is usually considered as the growth limiting factor because its concentration in the culture medium decreases rapidly. .[36-38,52-54]

As seen in Fig 7, the lowest multiplication rate is related to glucose- and protein-free culture medium. Based on the predictions made, the highest multiplication rate is in the MEM (complete medium).

BHK cells did not grow in the culture medium without protein. However, their situation was better than the medium without glucose. However the results show that the final number of cells has decreased compared to the initial cell number (multiplication rate less than 1). It was predicted that in the absence of protein, serum mainly formed from albumin and other proteins can compensate lack of protein in media and keep the cells alive and reach them to the proliferation phase but it is not occurred. Proteins act as carriers of molecules with low molecular weight and cause adhesion to culture surface.Albumin, transferrin, and fibronectin are the mostly found proteins in the culture medium. Albumin is the main protein of blood which is connected to water, salt, free fatty acids, hormones and vitamins and it is carrier of fats and fatty acids and displaces them between the tissues and cells. High binding capacity of albumin makes it appropriate for removing toxic substances from the cell culture medium. Fibronectin is a key ingredient in binding and adhesion of cells. Transferrin displaces iron and it is a protein transferring iron into the cellular membrane. It is an essential protein playing the major role of transferring iron into cells [40].

As it can be seen in Fig 8, the glutamine-free culture medium has the highest doubling time (43.79 hr). BHK cells grew and proliferated in the medium culture without essential amino acids, but this amount was not significant. Of course, it seems that absence of glutamine has the greatest impact among all essential amino acids in not progressing the cell culture process. Amino acids are the basic building blocks of proteins. Essential amino acids must be added to the culture medium because the cells themselves cannot synthesize them. They are necessary for cell proliferation and maximum concentration (large number) of cells. As in the other experiments, glutamine as an essential amino acid is of especial importance. Also, glutamine provides nitrogen for NAD, NADPH and nucleotides and acts as a secondary energy source for metabolism. Glutamine is an unstable amino acid and with time it turns into a state that cannot be used by the cells and thus should be added to the culture medium just before use [35, 54-57].Glutamine is the main source of nitrogen [36-37].Glutamine is an essential precursor for the synthesis of proteins and rib nucleotides. It is also an important respiratory fuel for rapid cell division and cells that do not have sufficient ability to use glucose [55-57].However, glutamine has two major problems: First is its instability and second is its degradation which produces ammonium. [58].

According to this Fig, the doubling time of glucose- and protein-free culture media cannot be calculated because the final number of cells has decreased compared to the initial cell number. but it show with zero in Fig.

The highest specific growth rate is for the MEM. The higher specific growth rate means the better conditions under which the cell culture has been done. (Fig.9)

Due to its importance in cell growth, glutamine is usually known as growth limiting factor because its concentration in the culture medium reduces rapidly. Glutamine consumption provides 30-65% of the energy required for reactions related to the mammalian cells [53, 55-57]. Although the absence of glutamine is problematic, its presence and also using by cells produces ammonium which is toxic to cells [38, 42].MEM with non-essential amino acids hasn't great effect on growth and proliferation of BHK cells.

The growth and proliferation of BHK cells in the culture medium without vitamin was very similar to their growth in the MEM culture medium. The results of this study show that the amount of vitamins synthesized by cells themselves and vitamins present in serum can greatly alleviates cells need for survival and proliferation. Vitamins are precursors for co-factors. Many vitamins are essential for cell growth and proliferation. Vitamins cannot be synthesized in sufficient quantities by the cells. Serum is a great source of vitamins used in cell culture. Vitamins of group B (riboflavin, thiamine, biotin, etc.) which are usually added to stimulate the growth are important for cellular biochemistry. At least seven vitamins including choline, folic acid, nicotine, pantothenate, pyridoxal, riboflavin and thiamin are important for cell growth and proliferation [29, 38-40, 45].

Table 3. The effect of supplements on the final cell number in suspension culture and with initial seed of $3.5*10^5$ and 5% PEG treated calf serum.

Experiment No.	The culture medium used	Number of cells at harvest per ml (Trypan blue) * 10000	Number of cells at harvest per ml (Nucleo count) * 10000
1	MEM	129±4	130±3
2	MEMWOGLUC[9]	19±2	20±3
3	MEMWOV[10]	113±3	111±4
4	MEMWOP[11]	22±3	22±3
5	MEMWOGLUT[12]	74±3	74±4
6	MEMWOEAA[13]	108±4	106±3
7	MEMWNEAA[14]	127±5	127±4

[9] Minimum essential media without glucose
[10] Minimum essential media without vitamin
[11] Minimum essential media without protein
[12] Minimum essential media without glutamine
[13] Minimum essential media without essential amino acids
[14] Minimum essential media with non-essential amino acids

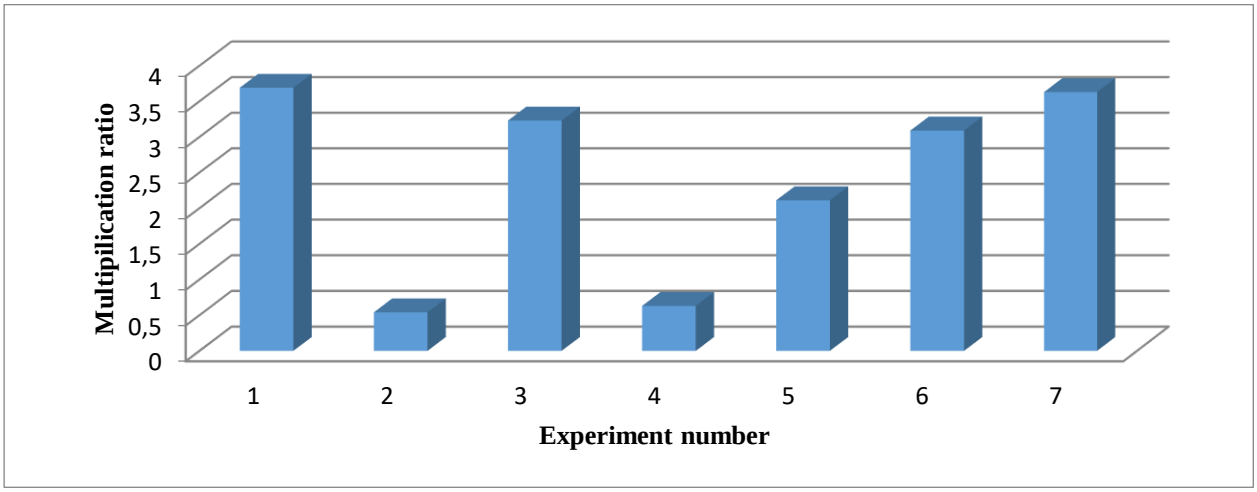

Fig. 7. ,Multipilication ratio in different media.(According to table3)

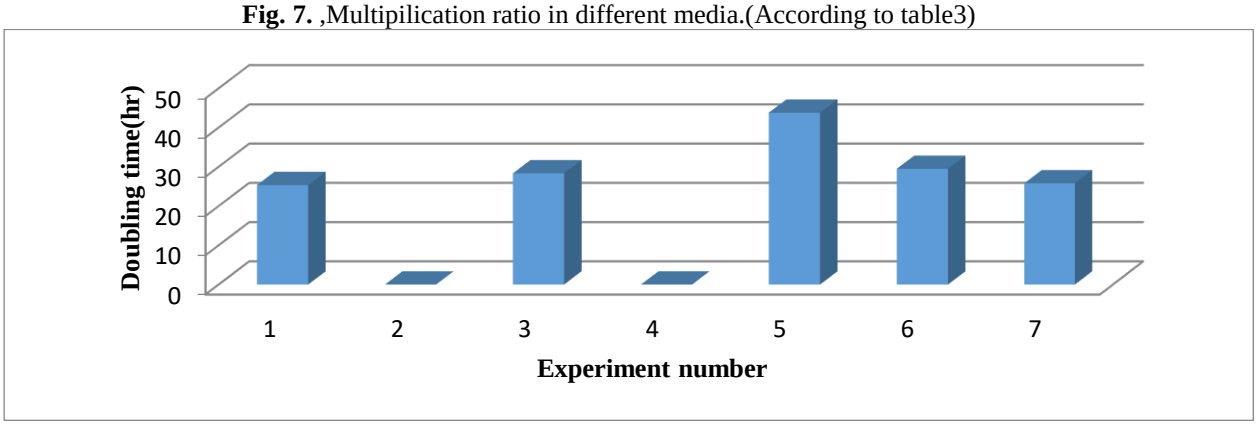

Fig. 8. Doubling time in different media.(According to table3)

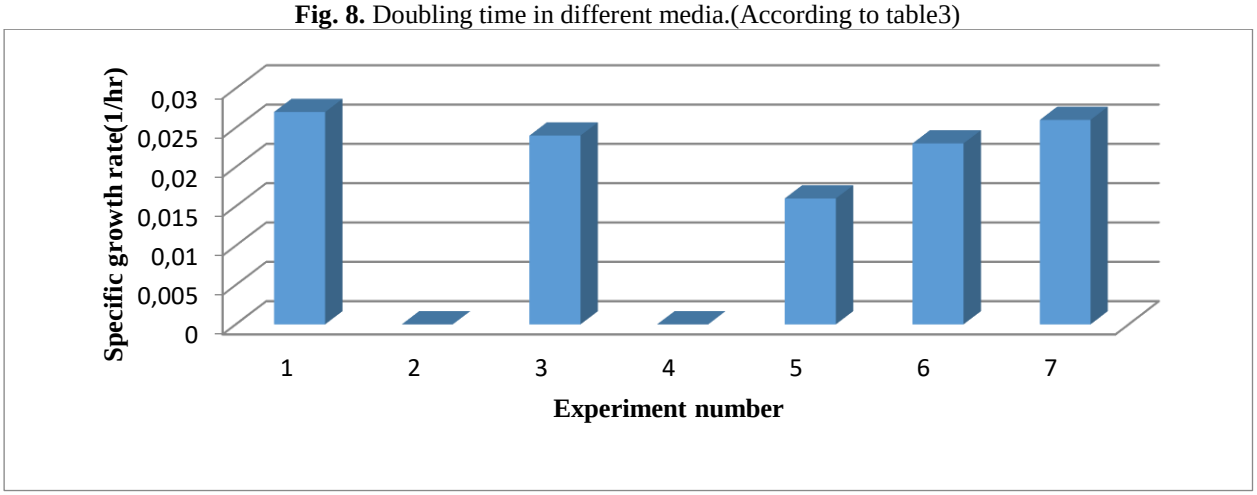

Fig. 9. Specific growth rate in different media.(According to table3)

CONCLUSION

Optimal amount of cell seed and serum, the presence of glucose, glutamine, proteins and vitamins are essential to achieve optimal growth and proliferation of BHK cells. The highest number of cells in the experiments performed in this study was obtained when all components of the culture medium were available and amount of serum and cell seed was appropriate. MEM media with protein in our study with 15% PEG treated calf serum has the best in result.

In this study it was shown that by increasing amount of cell seed and serum, we will not have more cells. Also, none of the components of the culture medium, especially glucose and protein, can be deleted and replaced with serum.

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