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Research article

EFFECTS OF FETAL BOVINE SERUM CONCENTRATION ON THE GROWTH AND SURVIVAL OF BHK 21 CELL LINES

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ABSTRACT: The aim of the present study was to study the effects of serum concentration on the Growth, Morphology and Viability of BHK 21 Cells. BHK Cells are widely used for production of various viral vaccines. Here two independent experiments were done with monolayers and BHK21 cells with control 10 % FBS constantly throughout the experiment, where as in test the serum concentration was reduced gradually up to 1.0 % and tried to adapt the cells at low serum concentration. ROS was measured in suspension cells by using H2DCFDA compound. The results were showed that reduce the cell count, Morphological changes, and increased the ROS in the in BHK 21 cells in test sample (under low serum concentration 2.0 % FBS).The results were statistically significant 'p' value is 0.001.

Key words: BHK21 Cells, FBS concentration, Cell growth and survival, ROS measurement.

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INTRODUCTION

Mammalian or Animal cell culture has got enormous applications in order to produce the desired products like recombinant proteins, Vaccines and others. Baby Hamster Kidney (BHK) Cells are originally derived from baby serian hamster (Mesocricetus auratus) kidney (Maceheron and Stoker., 1962).BHK Cells are generally adherent cell lines, it can also be used as suspension cell cultures.BHK Cells are widely used for production of various viral vaccines (Mowat, G. N et al.,1962, Hernandez et al., 2010 , Guo H et al., 2001), recombinant proteins) as well as stable and temporary transfections (Wurm FM, Bernard A, 1999). BHK cells can grow in a verity of media like Dulbecco's modified Eagle's medium DMEM, and Ham's F-12 medium, minimal essential medium, in house media supplied with all vitamins, amino acids, nutrients (Shekar P et al., 2008, Bradshaw GL et al., 1983,Raymond M et al, 2008). A typical culture medium is composed of a complement of amino acids, vitamins, inorganic salts, glucose, and serum as a source of growth factors, hormones, and attachment factors. Among them serum is one of the important nutrient for the growth of the cells. Without serum supplement it is difficult to survive the cells (Arora M et al., 2015, Diego L et al, 210).

Serum is a complex mix of albumins, growth factors and growth inhibitors (Yang H et al., 1991). Serum is one of the most important components of cell culture media and serves as a source for amino acids, proteins, vitamins (particularly fat-soluble vitamins such as A, D, E, and K), carbohydrates, lipids, hormones, growth factors, minerals, and trace elements. Serum from fetal and calf bovine sources are commonly used to support the growth of cells in culture (Clifford W et al., 1974). Fetal serum is a rich source of growth factors and is appropriate for cell cloning and for the growth of fastidious cells (Schumpp B et al., 1990) .

However, the cost of serum can account for 70-80% of the overall cost of the medium. Serum will also interfere in the purification of protein and vaccines (Hesham A, 2009).

Hence, the present study has under taken to see the possible reduction of FBS in cell culture for the reduction of cost of production and also see the effects of FBS on the growth of the BHK 21 cells. We have also made an attempt to measure the ROS produced by the cells during the adaptation of BHK 21 cells in low serum percentage.

MATERIALS AND METHODS

Cell lines

BHK 21 cells line were used for the whole experiments, kindly provided by Dr. Rabbani, King Saud University, Riyadh, Saudi Arabia.

Preparation of Media

Prepared Glasgow's Minimum Essential medium (GMEM) supplied (12.0g/L) with L- Glutamine and Phenol red. Sodium bicarbonate, Penicillin, Streptomycin, Kanamycin and peptone (Hi media) were added separately. Then adjusted the pH between 7.0 to 7.2.

Fetal calf Bovine Serum (FBS) was purchased from Bovogen (Australia). Phosphate buffer saline (PBS) and 2.5% of trypsin version solution (TVS) were prepared in house and adjusted pH 7.2 to 7.4.

Monolayer cell culture and adaptation in low serum concentration/percentage

BHK 21cells were maintained in a T75 flask with initial seed rate was 0.5 x 10^6 cells/ml in a 30ml Media with 10% FBS. Cells were grown on humidifier atmosphere, 37^0C, 5% CO2 incubator (Wilson et al., 2006). Here cells were grown in two flasks, one is control another is test. In the control BHK 21 monolayer cells were maintained constantly in 10% FBS throughout the experiment. But in Test Flask the FBS concentration decreased gradually. Initially with 10 % for 2 passages later 7.5, 5.0, 3.5, 2.0, 1.0%, it means gradually decrease the FBS concentration in the Test in order to see the effect of serum concentration on the growth and morphology of the cells. In each stage of the cells freezed and saved.

Suspension cell culture and adaption in low concentration/percentage

BHK 21 suspension cells were grown on shaker flasks with vented cap in a shaker incubator at 37^0C, 5% CO2 at RPM rate 125 to 140 RPM. For each passage 30 ml of media were used with 10% FBS in control at seed rate of 0.5 x 10^6 cells /ml. The suspension cells were prepared from monolayers. For better adaptation, first the cells were grown on mono layers with 10% FBS for 2 to 3 passages then theses cells are trypsinized with 0.25 % and added to suspension culture with 0.25% fluronic F-68, a surfactant was added in order to reduce the shear pressure between the cells and also reduce the foaming, while shaking the flasks. Later the serum percentage was reduced gradually like 7.5, 5.0, 3.5, 2.0 and 1.0 % in the test flask. For each passage kept 48 hours time duration to grow the cells.

Microscopic examination and cell enumeration

The cells were stained with Trypan blue (1:10 dilution) and Cell number was counted with Neubauer chamber under Compound Microscope (Olympus CX21) at every passage. The morphological changes of BHK21 cells were observed under microscope. The pictures of BHK cells were taken in inverted microscope attached with camera (Olympus).

Measurement of Reactive Oxygen Species (ROS) producing in BHK 21 cells by fluorescence spectro photometry.

Equal number of BHK21 (0.2 x10^6 cells/ml) cells were taken from suspension culture of 10% (Control) and 2.0% of FBS (Test) flaks. Cells were incubated with 30 µM dichlorofluorescindiacetate (H2DCFD) at room temperature for 30 min. Then the cells were centrifuged at low speed and wash the dye with PBS two times and suspended in 200 µl PBS. Fluorescent intensity was measured by (PerkinElmer, Waltham, MA) exited at 480 nm and emission spectra were collected from 490 nm to 620 nm (Danli W et al., 2011, Gomes A et al., 2005).

RESULTS

BHK 21 cells grown Monolayers at 10% FBS in showed normal in structure and shape (control. Fig 1A), where as in the test, cells were grown up to 3. 5% in normal structure .But from the 2% of FBS the cells were loss its structure and formed clumps. Moreover most of the cells became round shape and died as seen in the figure 1 B& C. For reliable results we have repeated the experiments 5 to 6 times till the consistent the results got .Student 'T 'test were done, It showed statistically significant 'p' value 0.001.

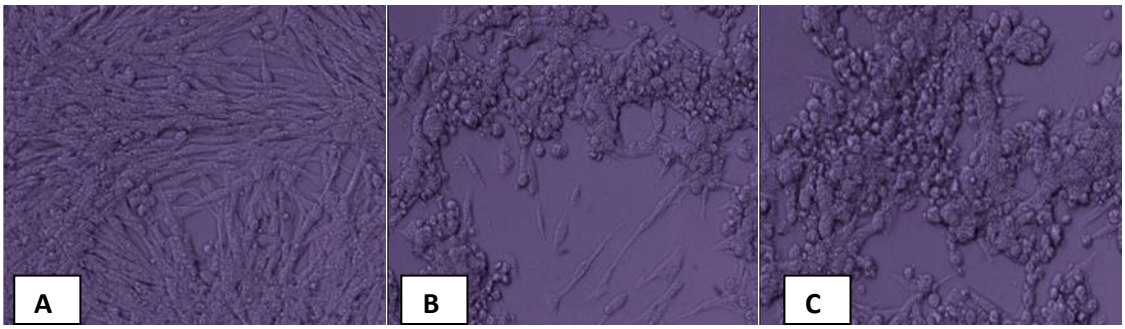

Fig-1. Microscopic analysis of morphological changes in BHK 21 Cells. A. BHK 21 cells were grown at 10% FBS (control) in Monolayers showing normal intact structure and shape. B. BHK 21 cells grown at 2.0 % FBS (Test) in Monolayers showing drastic changes in the morphology and most of the cells were died. C. BHK 21 cells grown on 1.0% FBS completely changed the morphology, appear as clumps and round dead cells.

BHK 21 Cells cultured as both monolayers as well as suspension cells. The BHK cells grown on monolayers in control has maintained normal cell count (0.5 X 10^6 cell/ml) as seen in the fig2a.Where as in test T75 flasks with different FBS concentrations (10,9.07.5,5, 3.5 2.0, and1.0%) as a monolayers as decrease the FBS concentration/percentage, decreased the cell count also . Each passage was grown for 48 hours .The cell count was 0.05 x 10^6 at 1% FBS. The Monolayers which were grown at low serum percentage like 2.0, 1.0 % FBS affect the cell viability up to 80-90%. The results were statistically significant, p value is 0.001(Fig 2a).

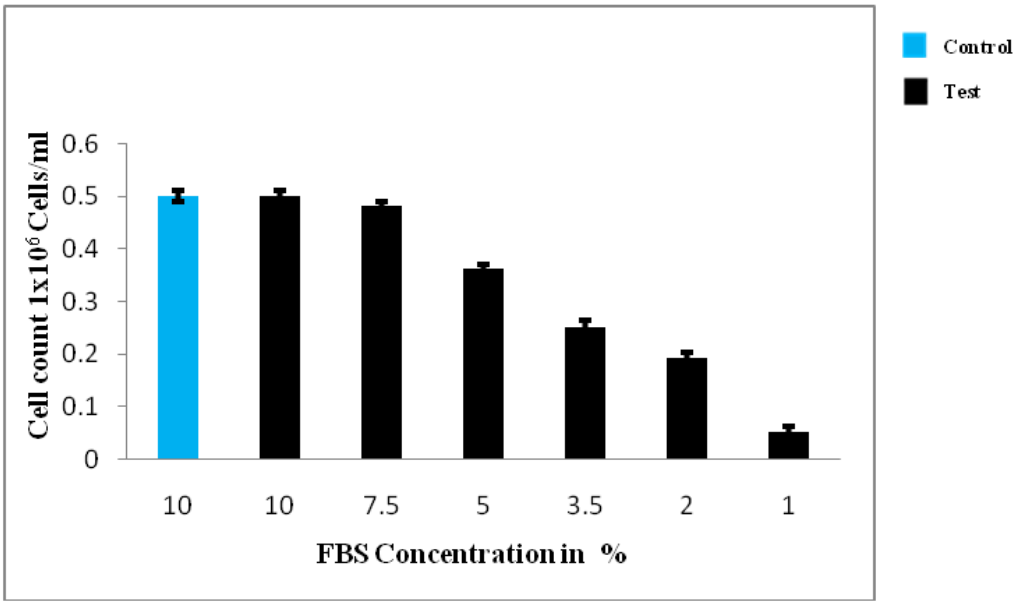

Fig-2a. Growth of BHK21 Monolayer cells in different concentration of FBS. (a)Monolayers of BHK 21 Cells cultured in T75 flasks with different FBS concentrations (10, 7. 5, 5, 3.5 2.0, and 1.0 %). It showed that gradual decrease in the growth of cells as decrease the FBS concentration. Each passage was grown for 48 hours. The Monolayers in 2.0, 1.0 % FBS, affect the cell viability up to 80-90%. Student 'T 'test was done, p value (<0.001) was significant.

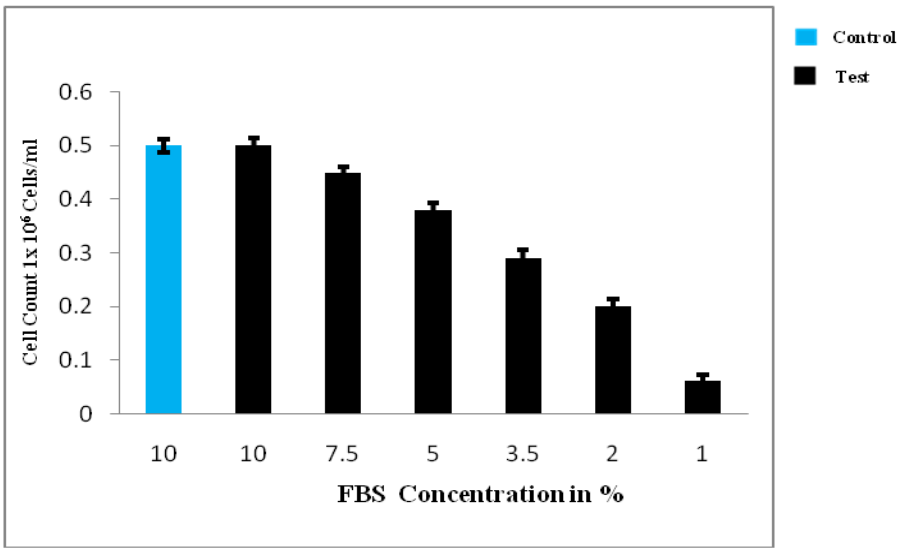

Fig-2b. Growth of BHK21 Suspension cells in different concentration of FBS. (b) BHK 21 Cells cultured in shaker flasks as suspension culture with different FBS concentrations (10, 7. 5, 5, 3.5 2, and 1%). It showed that gradual decrease in the growth of cells as decrease the FBS concentration. Each passage was grown for 48 hours. The suspension cells in 2,1 % FBS ,There is a loss of 80 to 90%% cell viability. Student 'T' test was done, p value (<0.001) was significant

BHK21 cells grown as suspension culture also done as two independent experiments as in monolayers. One is control with 10 % FBS showed normal growth of cells ($0.5x10^6$ Cells/ml) , another shaker flask (test) with different FBS concentrations (10,7.5,5, 3.5 2, and 1%). It showed that gradual decrease in the growth of cells as decrease the FBS concentration. Each passage was grown for 48 hours. Loss of 80% to 90%% cell viability and cell count was observed in th suspension cells in 1.0 % FBS, Student 'T 'test was performed and the results are statistically significant, p value (<0.001). Measurement of ROS in BHK 21 cells done using H2 DCFDA compound. Suspension cell culture of BHK21 cells were used for this experiment. In the control constantly supplemented with 10% FBS has also having some fluorescence, this is the normal generation of ROS during the metabolism. Where as in the test sample at 2% FBS produced more fluorescence than control (Fig.3). Fluorescent intensity was measured by exited at 480 nm and emission spectra were collected from 490 nm to 620 nm. The results were statistically significant.

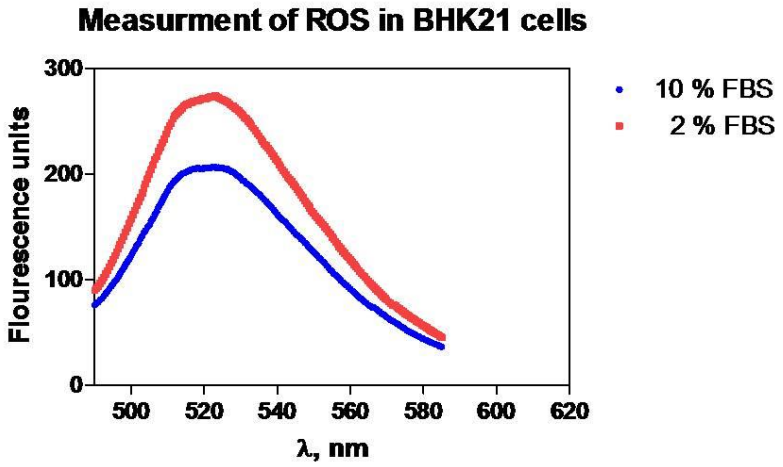

Fig-3. Measurement of ROS in BHK21 Cells. BHK 21 cells were incubated with 30 µM dichloro fluorescin diacetate (H2DCFD) at room temperature for 30 min. Fluorescent intensity was measured by exited at 480 nm and emission spectra were collected from 490 nm to 620 nm. It showed that more Fluorescence in 2 % FBS (Test) as compared to 10% FBS (Control) and is statistically significant (p value 0.001).

DISCUSSION

The results of the present study showed that the cell survival and morphology is decreased as decrease the serum concentration both in monolayers as well as in suspension cell culture. Previous studies also showed that FBS concentration may affect the cell growth, morphology and viability (Durrani A, et al., 2009, Hesham A, et al., 2009). Durani A et al, in 2009, showed that BHK 21 cells grown up to 1 % serum in low serum free media but the due to unaccepted morphological changes not advisable to consider the cells adapted with 1% serum. These results are in agreement with the present study. In our present study we did not use the serum free media only we observed the cell growth and morphology in the low concentration. The current study has also showed that as decrease the FBS concentration, increased the oxidative stress in the cells, there by decrease the cell growth and viability of the cells. Reactive oxygen species also measured and it showed that the cells at 2% of serum has more fluorescence by increasing ROS than cells growing in normal percentage of serum (10% FBS).This is because of starvation due to cells were grown on low serum concentration may induce the ROS, thereby increasing the fluorescence.

Many other studies were also tried to adapt the cells in serum free media in order to reduce the cost and purity of desired products like recombinant protein and vaccines and other products (Durrani et al, 2015, Hesham A, et al 2009, Diego et al, 2010, Mariani E et al., 1991, Barnes D et al., 1980).

However, Presence of serum in the media has many drawbacks and can lead to serious misinterpretations in immunological studies (Kerbel Ret al., 1976, Sula K et al., 1980). Hence, currently most of the scientists especially in industries are adapting the cells in serum free media to overcome all the problems.

CONCLUSION

The BHK 21 cells growth, viability and morphology were affected by reducing the serum concentration and also increased the Reactive Oxygen Species .The results were statistically significant p value is <0.001.

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