Comparative studies on media supplementation with various types of sera in tissue cultures

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Abstract

The present study was conducted to evaluate the growth promoting effect of different types of serum including; fetal calf serum (FCS), calf serum, sheep serum, goat serum, horse serum and African catfish serum on VERO cell cultures. These animal sera are used as nutrient supplements to VERO cell cultures. The evaluation of VERO cell growth was carried out by three techniques; turbid metric measurement, manual counts by hemocytometer and methylene blue dye reduction test. The data obtained by applying the three methods of evaluation revealed that, VERO cells proliferation in tissue culture media containing the different tested sera with different concentrations was variable. FCS had the best growth rate followed by calf serum, goat serum and African cat fish serum. Horse serum and sheep serum had the least growth rates among all of the tested sera. Hence, African cat fish serum can be used as an alternative to overcome the disadvantages of other sera.

Keywords: Sera – VERO cells – Fetal calf – Calf – sheep – Goat – Horse – African catfish

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Introduction

Serum, a common used supplement to cell culture media in most of mammalian cell cultures .that is added in the range of 5 – 15 % (v/v)) to promote and sustain cell growth as well as provide buffering and protection to cells [1]. Serum is known to provide the media with a broad spectrum of macromolecules, carrier proteins for lipoid substances, trace elements, attachment

and spreading factors, low molecular weight nutrients, hormones and growth factors, which are essential to most types of cell cultures. Serum can be obtained commercially and being sourced from a variety of species such as bovine, chicken, caprine (goat), equine, ovine, porcine, rabbit and even human [2]. Among all of variable sources, Fetal calf serum (FCS) is the serum of choice in mammalian cell culture [3, 4] because of its components of growth-promoters specifically, 30 – 50 % (v/v) bovine serum albumin[4] .A result of supporting multiplication of most cell types, fetal calf serum (FCS) has been considered as the most important cell culture supplement. Its growth promoting activity is better than adult bovine or bovine calf serum[5]. In spite of; it is rich in growth factors, vitamins, hormones and other nutrients that are essential for proliferation of mammalian cells. Fetal bovine serum is low in total proteins and immunoglobulin contents [5]. Apart from all of the benefits in FBS it could be complicated by many health concerns such as BSE (bovine spongiform encephalitis), FMD (foot and mouth disease) and rinderpest, to name a few [2].

Ahmad et al reported that on comparing the use of the locally made bovine serum from Halal slaughtered house with the commercially available serum GIBCO that is used in Vero cell cultures. He found that the maximum viable cell numbers were obtained when culture was supplemented with locally produced bovine serum than that's supplemented with commercial bovine serum after four days[6] .Serum from donor horses is also available in the market. It's passed with pioneered processes that guarantee high quality donor horse serum. In addition the collection of blood from horse donor was claimed to be stress-free [7] .The use of horse serum as a cell culture media supplement was evaluated at various concentrations, so it was found that high concentration (20% or more) favored cell attachment but inhibited cell proliferation and differentiation. However, lower concentrations (5% to 10%) favored cell proliferation and differentiation [8].

Goat serum was found to be suitable for most of the cell lines and primary cultures. It was observed that various types of Primary cultures of guinea pig embryo, monkey kidney, chick embryo and mouse peritoneal macrophages, when grown in a growth media supplemented with goat serum, showed established cell lines[9] .Goat serum could be used as effectively as newborn calf serum for routine culture of fish cells at a concentration of 10% [10].

Concerning fish serum, it can be successfully used as media supplement for cultivation and maintenance of primary cell culture from fishes [11, 12] .Dulbecco's modified Eagle's medium (DMEM) supplemented with either 10 or 20% FCS (fetal calf serum) or 5% carp fish serum (CFS) showed the best cell density[13] .Fish serum from surimi wash water processing line was reported as a potential substitute for serum in mammalian hybridoma cell culture[12]. It was found that in all substituted cultures, the cells produced antibody until the third passage; however, the higher antibody secretion was obtained on substitution of 2% FBS with fish serum especially in cultures maintained for 96 hours after the third passage[12].Hence, many trials have been made to find another source for medium supplement instead of FBS to get over the problem of microbial contaminants. Therefore sights have been directed to fish as a source of nutrient medium,

because until now no DNA or RNA viruses infecting fish have been reported to infect humans and animals[14]. Using fish serum in mammalian cell culture could be safer than fetal calf serum (FCS) [15]. However, the stimulating activities of FS for the adhesion and proliferation of most types of cell cultures are unknown and need to be investigated.

Aim of the work

The present study was constructed to evaluate the potential substitution of FCS by another serum type in culture media.

Materials and methods

Materials

A - Different sera

The sera used in the present study were Calf serum, Fetal Calf serum, equine serum, sheep serum, goat serum and Nile Catfish serum. The concentration which was used from each serum type was approximately 2- 15%. Only, commercially fetal calf serum (Gibco – USA) (virus and mycoplasma screened) was used as supplement for cell culture media while the other sera types were prepared.

B - Tissue cultures

Vero cells were seeded at a density of 2 ×104 cells/cm2 into prescription bottles. Then the inoculated bottles were incubated at a temperature of 37 °C.

C - Tissue culture media

Dulbecc's MEM was used for propagation and maintenance of VERO cells. It was prepared according to the manufacturer's instructions. The medium was supplemented with different types of sera with concentration 2 %, 4%, 6%, 8%, 10 %, 12% and 15 %.

Methods

A – Preparation of serum samples:-

We Collected 500 ml of each of calf blood, horse blood, sheep blood and goat blood aseptically from animals belonging to SVRI, Abbasia, Cairo ,Egypt . The collected blood was left at room temp for 30 minutes (min) for clotting. The clear serum was centrifuge at 2000 rpm for 10 mints at 4°C. Then the supernatant was collected into sterile six 30 ml corex tubes, further was centrifuged at 10,000 rpm for 10 min at 4°C. The complement components were inactivated

by keeping the serum bottles in a water bath at 56°C for 30 min, then serum was left to come to room temp. After that, the serum was sterilized, then transferred into sterile serum bottles and stored at – 20 °c for further use[16] .Fish serum was prepared by the same steps after withdrawal of fish blood from tail vein of North African catfish (Nile Catfish) [17].

B - Evaluation of tissue culture growth:-

The growth of VERO cells in tissue culture media supplemented with the previously prepared different types of sera with different concentrations was evaluated by three different methods.

1 – Indirect cell counting by spectrophotometer (Turbid Metric Measurement)

Indirect counting of tissue culture growth by using V-530 UV/VIS spectrometer, code number 6736-J004A, Jasco International Co., Ltd. The optical density (OD) is directly proportional to the biomass in the cell suspension in a given range that is specific to the cell type. Using spectrophotometry for measuring the turbidity of cultures recording the absorbance values (OD) under 570nm wavelength. This method is known as turbidometry. Spectrophotometry's obstacle was the inability to provide a differentiation between living and dead cells[18].

2 -Manual counts by hemocytometer

A clean, sterile hemocytometer was used; a coverslip then gently placed on the top of counting chamber. By gentle pipetting, 50 µL of sample was mixed with 50 µL of 0.4% trypan blue, and then 20 µL of the mix were loaded into each chamber of the hemocytometer to enter the counting chamber by capillary action. Trypan blue was added to the cell suspension (in a 1:1 ratio). During determination of cell viability, separate counts for both live and dead cells were made, the dead cells appearing blue due to the permeability of their damaged membranes to trypan blue. The microscope is then focused on an area of the counting chamber and the cells were counted using a tally counter. The concentration of viable cells / ml = average of cells per square × dilution factor $\times 10^4$ [19].

3 – Assessing of tissue culture by methylene blue dye reduction test

Methylene Blue Dye Reduction Test, commonly known as MBRT test, it is used as a rapid method to evaluate the tissue culture growth. This test is based on adding the dye solution to the media; its blue color gets decolorized when oxygen present in the media gets exhausted as a result of the growth activity of tissue culture. The test has to be done under sterile conditions. We added 1 ml methylene blue dye solution (dye concentration 0.005%) to each 10 ml of tissue culture media in the prescription. recording this time as the beginning of the incubation, followed by recording the reduction in color by spectroscopy at a wavelength of 570 nm[20].

Statistical analysis

The data was analyzed by ANOVA to determine the significance of differences among means [21].

Results

To gauge the influence of each type of serum used in this study on the growth of VERO cell tissue culture. VERO cells were cultivated for 72 hours in media containing different concentrations from each serum included in this study. The concentrations of sera used in this study were 2% ,4% ,6% ,8%,10% ,12% ,15% .The evaluation of growth of VERO cell culture were done using three techniques, turbid metric measurement, manual counts by hemocytometer and methylene blue dye reduction test.

The data obtained by the three methods of evaluation in the present study revealed that; VERO cell proliferation in tissue culture media containing the different tested sera with different concentrations was variable among all as will be described later. Regarding the analysis of results obtained from turbid metric measurement, these results showed that there was positive effect through increasing the concentration of FCS on the proliferation of VERO cells. The best results obtained were on using 15% FCS where its optical density was (0.530±5) ($p<0.05$), 15% sheep serum where its optical density was (0.420±5) ($p<0.05$), 12% goat serum where its optical density was (0.440±5) ($p<0.05$) 15% equine serum where its optical density was (0.365±5) ($p<0.05$). 12% calf serum where its optical density was (0.450±5) ($p<0.05$) and 12% African catfish (Nile Catfish) serum where its optical density was (0.410±5) ($p<0.05$) as shown in table (1) and chart (1).

The previously mentioned results of turbid metric measurement were more or less compatible with the results of the other two methods. Concerning the results of hemocytometer, it shown that; the highest total number of living cells logged on using 15% fetal calf serum (FCS) were (4.8×10^4) ($p<0.05$). Conversely the lowest total number of living cells reported by this technique was on using 15%serum of equine (2.9×10^4) ($p<0.05$) and 10% sheep serum (3.2×10^4) ($p<0.05$). High total number of living cells was noted on using 15% sera of calf, goat and African catfish (4.3×10^4) ($p<0.05$), ($4,2\times10^4$) ($p<0.05$) and (3.9×10^4) ($p<0.05$) respectively as showed in table (2) and chart (2).

In regards to methylene blue dye reduction test the results revealed that the high rate of reduction of methylene blue dye was recorded on using 15% fetal calf serum (0.360) ($p<0.05$) and the lowest reduction rate to methylene blue dye recorded on using 15% equine serum (0.520) ($p<0.05$) . Moreover the high reduction to methylene blue was noted in case of sheep and African

catfish serum was at 10% (0.510) ($p<0.05$) and (0.430) ($p<0.05$) respectively , as well as in case of 15% goat and calf serum was (0.450) ($p<0.05$) and (0.430) ($p<0.05$) respectively as shown in table (3) and chart (3).

Table (1): The effect of using different concentrations of serums on growth of VERO cells after being cultivated for 3 days using turbid metric Measurement

Concentrations of Serum / Type Of serum	2%	4%	6%	8%	10%	12%	15%
Sera of sheep	0.170	0.230	0.280	0.330	0.380	0.390	0.420
Sera of goat	0.180	0.260	0.310	0.380	0.410	0.440	0.430
Sera of equine	0.130	0.280	0.300	0.310	0.340	0.360	0.365
Sera of calf	0.140	0.230	0.320	0.355	0.410	0.450	0.440
Sera of fetal calf	0.230	0.360	0.410	0.430	0.490	0.520	0.530
Sera of African catfish (Nile Catfish)	0.260	0.320	0.350	0.360	0.390	0.410	0.410

*Treatment means did not differ significantly ($P< 0.05$)

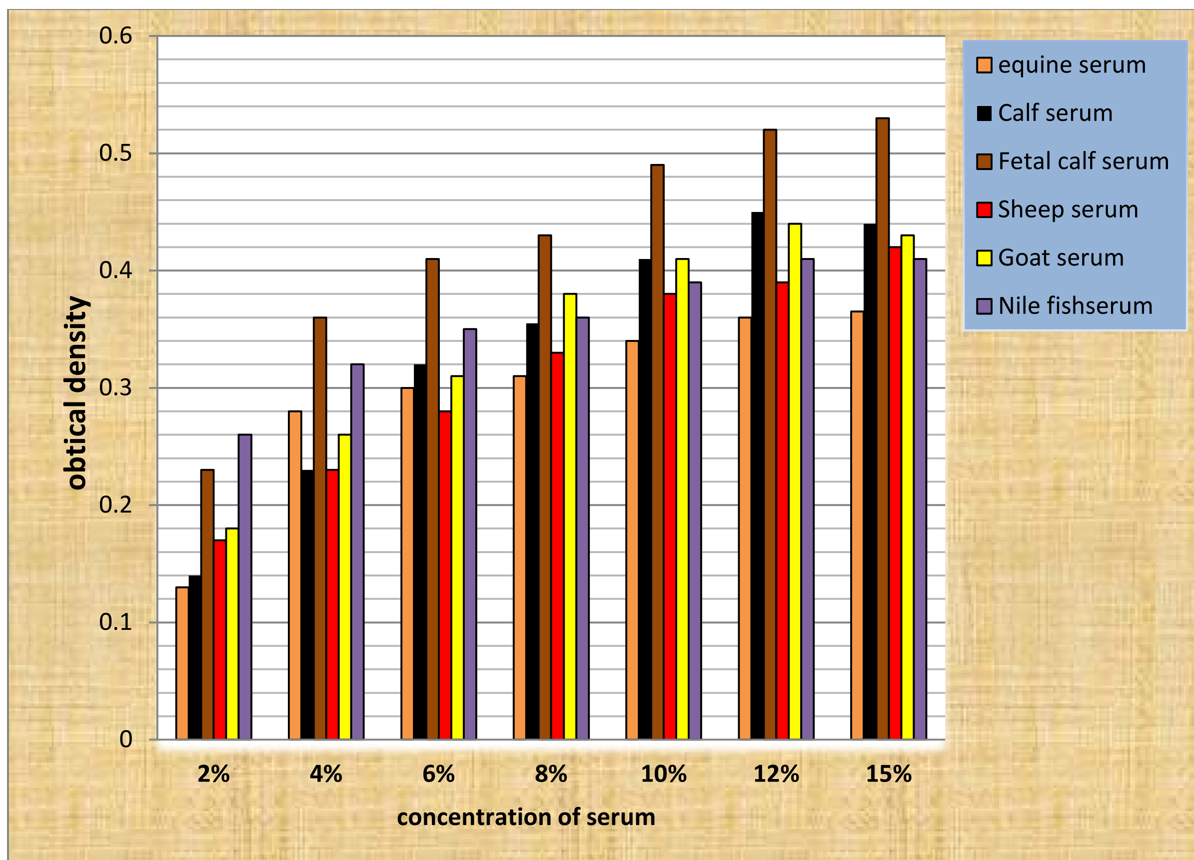
Discussion

Many trails have been made to find other sources for nutrient media instead of fetal calf serum (FCS), bovine serum, equine serum, goat serum and sheep serum to overcome cross-reaction and microbial contamination. The present study evaluated the effect of media containing different concentrations of various types of sera on the growth of VERO cells for 72 hours. The evaluation was carried out by different three methods (turbid metric measurement, manual counts by hemocytometer and methylene blue dye reduction test).

Our results of the different three methods showed that; the highest rate of VERO cells growth was observed on using 15% concentration of fetal calf serum followed by newly born calf, goat

and African catfish sera. While 15% of sheep and equine sera showed the least growth of VERO cells. In addition, it was noted that the rise in serum concentration in the growth media, upsurges in cell density. These results partially agreed with those of Rahman et al, [22] who concluded that FCS was the best growth promoting agent for BHK-21 cell line followed by goat, sheep and calf sera.

Chart (1) the effect of using different concentrations of serums on growth of VERO cells after being cultivated for 3 days using turbid metric Measurement.



*Treatment means did not differ significantly (P< 0.05)

The highest growth rate on using FCS could be due to its contents of nutritional and macromolecular growth factors essential for cell growth[9]. In addition, fetal calf serum can support the multiplication of most cell types. The growth promoting activity of fetal calf serum is better than that of bovine calf serum due to its contents of immunoglobulin, growth factors, vitamins, hormones and other nutrients that are essential for proliferation of mammalian cell [5].Moreover, the present study demonstrated that; 6-10% FCS can be used for the maintenance and persistent cultivation of VERO cells culture. These outcomes are congruent with [23, 24] both

studies suggested the use of 5-10% fetal calf serum for maintenance and persistent cultivation of all types of adherent cell cultures. Furthermore, our results of the three methods of evaluation revealed that sera of sheep, goat, bovine calf, equine and African catfish had variable growth supporting ability for VERO cell culture. These finding are parallel with those mentioned by [25, 26,22,12,15].

Table (2): The effect of using different concentrations of serums on growth of VERO cells after being cultivated for 3 days using hemocytometer

Concentrations of Serum / Type Of serum	2%	4%	6%	8%	10%	12%	15%
Sera of sheep	1.6	2.1	2.8	3.1	3.2	3.1	3.2
Sera of goat	1.7	2.4	2.9	3.3	3.6	3.7	4.2
Sera of equine	1.4	1.6	2.1	2.5	2.7	2.7	2.9
Sera of calf	1.6	2.3	2.9	3.4	3.8	4.1	4.3
Sera of fetal calf	2.3	2.7	3.3	3.9	4.3	4.7	4.8
Sera of African catfish (Nile Catfish)	2.7	2.9	3.3	3.4	3.8	3.8	3.9

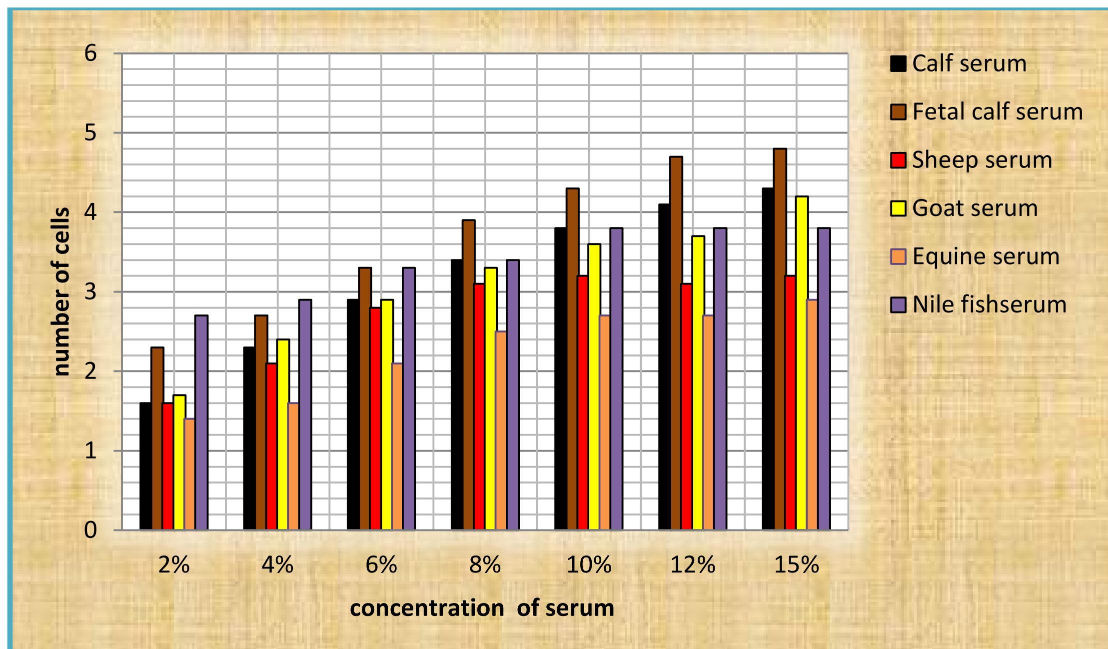
*Treatment means did not differ significantly ($P < 0.05$)
**This values are multiplied by 10^4.

In regard to results of hemocytometer; it is considered as the most accurate, due to its evaluation of the living cells only. It showed that the highest total number of living cells logged by using 15 % fetal calf serum followed by using 15% sera of bovine calf , goat and African catfish. From these results we can state that both of African catfish serum and goat serum can be used instead of fetal calf serum and bovine calf serum. These results are in agreement with those of [27, 9,12,15,10].

Our results regarding goat serum showed that; serum of goat is as suitable to VERO cells growth as calf serum and to some extent FCS , a result that is matching with that of [9], who

mentioned that; goat serum is considered appropriate for major types of cell cultures and primary cultures from guinea pig embryo, monkey kidney, chick embryo, mouse peritoneal macrophages, and established cell lines were prepared and grown in growth media supplemented with goat serum. These cultures were studied for their morphology and growth in comparison with cultures grown in FBS containing media.

Chart (2): The effect of using different concentrations of serums on growth of VERO cells after being cultivated for 3 days using hemocytometer.



*Treatment means did not differ significantly ($P< 0.05$)
**This values are multiplied by 10^4.

It is well known that; sera of fetal calf, bovine calf, sheep, equine and goat could be a potential source of bacterial, viral and mycoplasma contamination e.g. bovine spongiform encephalopathy (BSE) and mycoplasma[28,29,30]. Contamination of cell cultures because of infectious organisms in serum could constitute a serious problem. Bacteria, fungi, viruses and mycoplasma have been isolated from bovine serum. In the period from 1960 1980, mycoplasma from bovine serum was the second major group of contaminants found in cell culture[31]**.**Now; FBS is usually screened for mycoplasma and most viruses. However, a more serious cause for concern is all protein infectious agents called a prion for which no test is available[32]. This organism causes a fatal brain disease in mammals called Bovine Spongiform Encephalopathy (BSE), or "mad cow disease". BSE occurs in sheep, cows, and other mammals, and is most likely

the cause of similar neuro-degenerative diseases in humans. So that sights have been directed to fish as a source of nutrient medium in mammalian cell cultures. To date no DNA or RNA viruses infecting fish have been reported to infect animals and human[14] thus; employing fish serum (FS) in mammalian cell culture for medically related use could be safer than employing FCS[15].

Table (3): The effect of using different concentrations of serums on growth of VERO cells after being cultivated for 3 days using methylene blue day reduction test

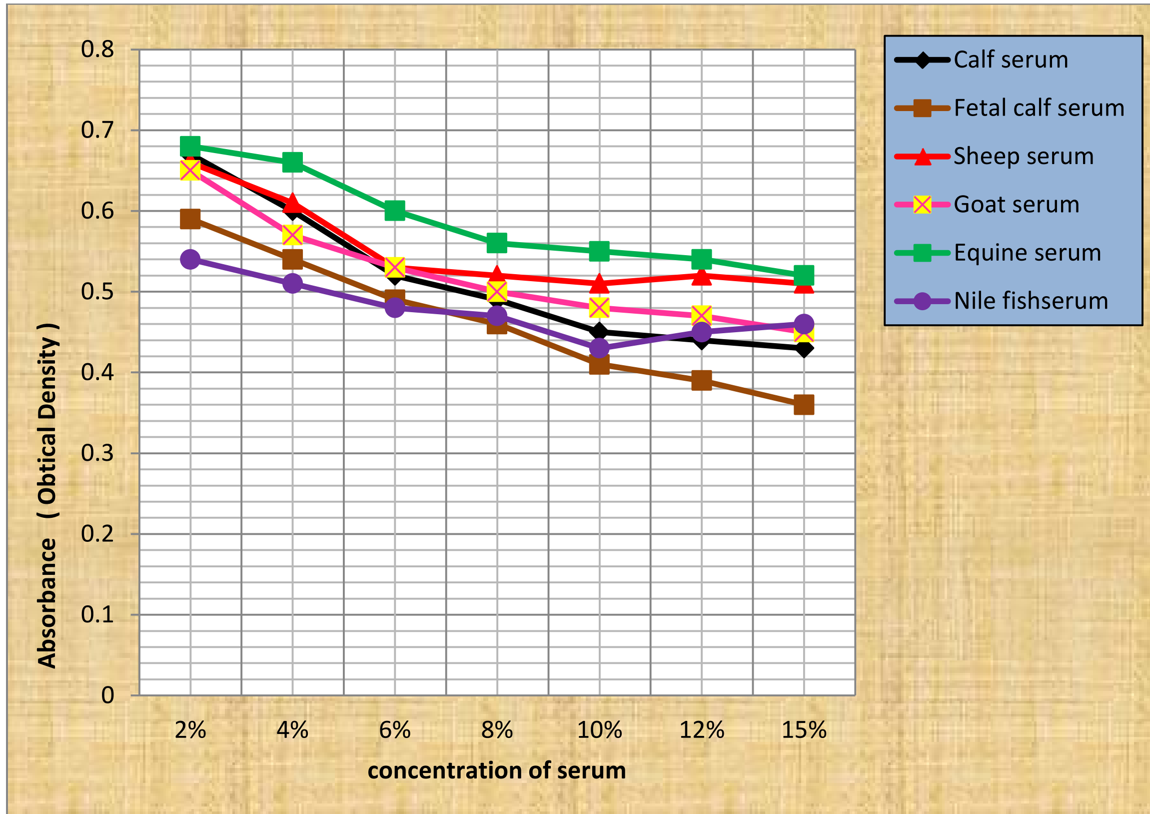
Concentrations of Serum / Type Of serum	2%	4%	6%	8%	10%	12%	15%
Sera of sheep	0.660	0.610	0.530	0.520	0.510	0.510	0.510
Sera of goat	0.650	0.570	0.530	0.500	0.480	0.470	0.450
Sera of equine	0.680	0.660	0.600	0.560	0.550	0.540	0.520
Sera of calf	0.670	0.600	0.520	0.490	0.450	0.440	0.430
Sera of fetal calf	0.590	0.540	0.490	0.460	0.410	0.390	0.360
Sera of African catfish (Nile Catfish)	0.540	0.51	0.480	0.470	O.430	0.450	0.460

*Treatment means did not differ significantly (P< 0.05)

Conclusion

The sera of fetal calf, calf, sheep, goat, equine and African catfish can be used as nutrient supply. We prefer using African cat fish serum to overcome cross reactivity, infectious organisms and protein infectious agents called a prion.

Chart (3): The effect of using different concentrations of serums on growth of VERO cells after being cultivated for 3 days using methylene blue day reduction test.



*Treatment means did not differ significantly (P< 0.05)

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