Investigation the role of fetal calf serum on the viability of gonad cells cultured in vitro for fresh water snail *bellamya bengalensis*

التحري عن دور مصل جنين العجل على حيويه الخلايا التكاثريه المزروعه لقوقع المياه العذبه بللاميا بنكالينسس خارج الجسم الحي

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Abstract

The present study conducted to investigate the role of fetal calf serum (FCS) on the viability *in vitro* cultured gonads cells of fresh water snail *bellamya bengalensis* and determine the optimal concentration of (FCS) for gonads cell cultured *in vitro*. the fresh water snails *bellamya bengalensis* were collected from Shamyiah river-Dewanyah province, the snail were dissection out to obtain the gonad cells ,which cultured in (M199 medium) medium supplemented with different concentration of fetal calf serum (10%,20%),the gonad cells were cultured in (M199 medium) supplemented with (FCS) revealed higher viability rate compare with basal medium(non-supplemented with FCS) and control ,while the medium supplemented with (20% FCS) had more positive effect on the viability on the viability of gonad cells than the medium supplemented with(10% FCS). FCS had positive effect to maintain the viability of gonad cells for *bellamya bengalensis in vitro* cultured .

Key words: FCS , M199 medium

الخلاصة:

هذه الدراسه صممت للتحري عن دور مصل جنين العجل على حيويه الخلايا التناسليه لقوقع المياه العذبه ***بللاميا بنكالينسس*** المزروعه خارج الجسم الحي,وتحديد التركيز الأمثل لمصل جنين العجل للخلايا التناسليه المزروعه خارج الجسم الحي. تم جمع القوقع من نهر الشاميه –محافظه الديوانيه ومن ثم تشريح القوقع للحصول على الخلايا التناسليه التي زرعت في الوسط الزرعي(M199)و الوسط الزرعي (M199) مدعم بتراكيز مختلفه من مصل جنين العجل(10%و 20%). الخلايا التناسليه المزروعه في الوسط الزرعي (M199) المدعم بمصل جنين العجل أظهرت معدل عالي من الحيويه مقارنه بالوسط الزرعي(M199) الغير مدعم بالمصل ومجموعه السيطره, بينما الوسط الزرعي (M199)المدعم ب(20%) من مصل جنين العجل كان له تأثير أيجابي أكثر على حيويه الخلايا التناسليه المزروعه مقارنه بالوسط الزرعي (M199) المدعم ب(10%) من مصل جنين العجل .مصل جنين العجل كان له تأثير أيجابي في أدامه حيويه الخلايا التناسليه المزروعه لقوقع المياه العذبه ***بللاميا بنكالينسس*** خارج الجسم الحي

Introduction

In cell and tissue culture procedures which needing to use animal sera products , routinely fetal calf serum (FCS), as culture medium supplements ,FCS is easily taken and consist of a lot of growth factors, compared to other animals sera (1).This reason lead to consider that FCS as global supplement for *in vitro* cell and tissue culture media ,the standard concentrations of FCS that using it as supplement in cell and tissue culture media ranged from 5% to 20%, the composition of FCS consist of different components such as (proteins , vitamins, , lipids, hormones proteins ,binding factors, detoxifying agents, proliferation factors, and growth factors) (2).

FCS have been used in different cultures media as a nutritional factor, attachment factor and growth factors essential for cell proliferation and maintain cell viability (3).

A lot of cell cultures media have been synthesized to contain serum, serum stills to be used in cell and tissue cultures media by many approaches (4).

The most routinely used as supplement added to different cultures media was fetal calf serum (FCS), a lot of sort of cell cultures have been own the optimal concentration of FCS, demand for saving of various cell lines *in vitro* (5). The present study aimed to: investigate the growth

promoting effect of FCS and its optimal concentration for the maintenance the viability and prolonged cultivation of gonads cells of fresh water snails *Bellamya bengalensis in vitro* cell culture laboratory of our own local environment.

Material and methods.

Sample collections

Fresh water snails of the species *Bellamya bengalensis* (average size of 2.5 cm) were collected from Shamyiah river in Dewanyah province in April 2013 by simple hand picking method ,freshly collected specimens were carried to the laboratory of college science –Qadisyah university and placed in aquarium and half of fresh water in the aquarium was renewed every two days, the animals was fed with lettice and introduced to analysis. Then the specimens dissected out, and the gonads of have been obtained carefully and gently to prepare them to cell culture studies (6).

The gonad cell culturing using culture media.

The Gonads explant from the *Bellamya bengalensis* were dissected out into small cellular fragments and placed in petri dish containing 2ml fresh water and the petri dishes were stirred during this period in order to make easier the fragments disassociation. From the petri dish the suspension was taken out and put in a 15ml Falcon tube. The suspension was centrifuged at 15°C, 150 xg, for 4 minutes, the supernatant was discarded and the pellet of cells resuspended in 2mL of fresh water . Using the same parameters the pellet of cells were resuspended in 2mL of cell culture media (7,8).

The cell suspension was transferred in a multi-wells and left at 15°C. Cells viability was checked every three days by using trypane blue dye (9) ,the culture medium was substituted tow times per week order to avoid possible contamination (7,8).

Results

This study designed to investigate the effect of fetal calf serum (FCS) supplemented to M199 medium on the viability of gonads cells, we used tow concentration of serum 10% and 20%.

Our results pointed out that, there was significant differences($p \leq 0.05$) between M199 medium supplemented with FCS and M199 medium at the first day ,when the viability of gonad cells reached to 93% in M199 supplemented with 10% FCS and 95% in M199 medium supplemented with 20% FCS compare with 82% in M199 and 3% in control .but no significant differences($p \leq 0.05$) between the viability of gonad cells in M199 supplemented with 10% FCS and M199 supplemented with 20% FCS.

Also the results reported that ,there was significant differences($p \leq 0.05$) between the viability of gonad cells in in M199 supplemented with 20% FCS M199 supplemented with 10% FCS, M199 medium and control at the third day ,when the viability was (86,81,74,0)% respectively.

At the sixth day ,there was significant differences($p \leq 0.05$) between the viability of gonad cell in the culture media ,when the viability was (78,73,63,0)% in M199 supplemented with 20% FCS, M199 supplemented with 10% FCS, M199 medium and control respectively.

At the ninth and twelfth days, there was significant differences ($p \leq 0.05$) between the viability of gonad cell in the all culture media ,when the viability was (54,44,33,0)% at the ninth day and (36,22,0,0)% at the twelfth day in in M199 supplemented with 20% FCS, M199 supplemented with 10% FCS, M199 medium and control respectively.

At the fifteenth day there was clear significant differences($p \leq 0.05$) between the viability of gonad cell in the all culture media ,when the viability was (21%) in M199 media supplemented with 20% FCS ,while the viability was(0%) in , M199 supplemented with 10% FCS, M199 medium and control(table3,figure1).

Viability %

Culture Media	1 day	3 days	6 days	9 days	12days	15 days
Control	3	0	0	0	0	0
M 199	82	74	63	53	0	0
M 199 + 10 % FCS	93	81	73	44	22	0
M 199 + 20 % FCS	95	86	78	54	36	21

Table3:The viability of gonad cells cultured in M199 medium,M199 medium supplemented with FCS.

L.S.D ($p \leq 0.05$):6.2

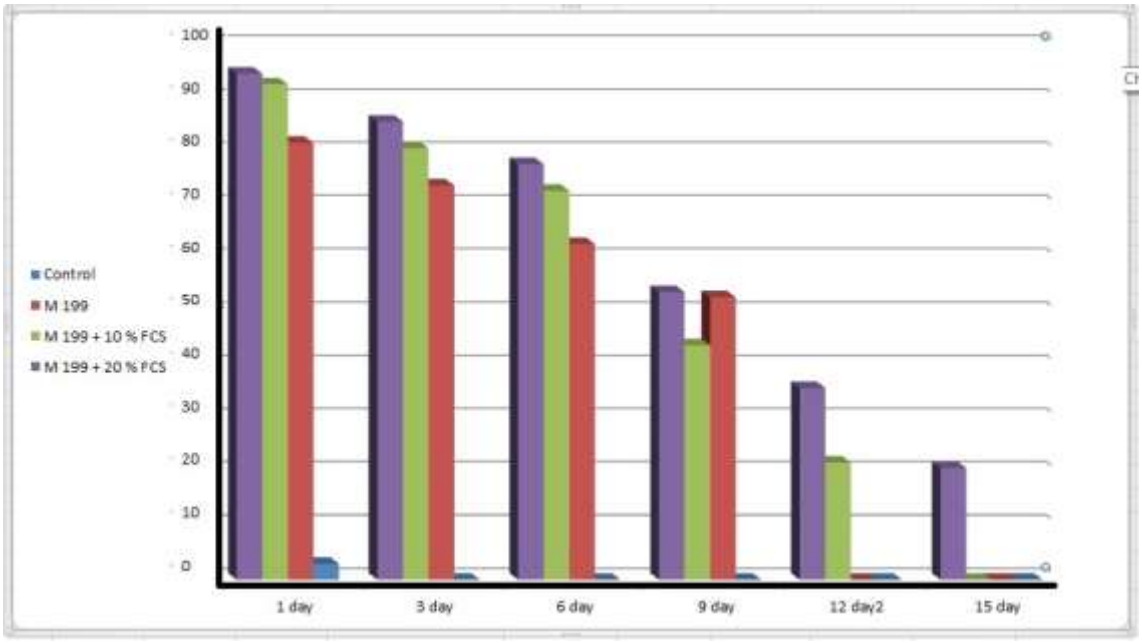

Figure 1:comparsion between the viability of gonad cells in M199medium and M199medium supplemented with different concentrations of FCS.

Discussion

During the initial development of the cell culture *in vitro* the medium was supplemented with fetal calf serum (FCS) to enhance growth of culturing cells and maintain the cells viability (10). According to all animal sera, fetal calf serum was known to improve the *in vitro* cells culture of a lot of cell types including fresh water invertebrates .The ability to have proliferation -promoting effects might the (FCS) had a lot of mediators and to growth factors, which contained in FCS (11).

In this papers, In order to improve cell culture techniques, the M199 medium supplemented with different concentrations of FCS in experiments performed with gonad cells of *bellamya bengalensis*. The main objective of the present work was to evaluate with a quantitative approach the effects of different FCS concentrations supplemented in the M199 medium used for snail gonads cell culturing *in vitro*. Our results showed that the cells cultured in M199medium supplemented with fetal calf serum (FCS) level of 20% was optimal, in this level we got high rate of cells viability ,and the culture media kept the gonads cell still a live over two weeks , under this condition, the gonads cell cultures reached a maximum ratio of viability , this findings agree with(12) who regards that basal culture media supplemented with FCS had positive effect on the cell proliferation and maintain the viability compare with basal media without supplements.

The results of our study also agree with previous studies has been reported that the use of fetal calf serum at concentrations of 10–20% has been reported to be beneficial effect for fresh water invertebrates cell cultures *in vitro* (13,14). When fetal calf serum has been used as a supplement to basal growth medium in this study (M199), it has been demonstrated that the components of fetal calf serum such as (proteins, hormones, lipids and growth factors) stimulated cell growth and proliferations and maintain the viability of gonad cells, but our results dis compatible with (15) they demonstrated that the fetal calf serum had less influence when use as supplements in mammalian cell cultures.

it was well demonstrated in the present study that the increase in fetal calf serum (FCS) level from 10% to 20% had positive effect on cells growth and maintain cells viability, but the most effect was M199 supplemented with (FCS) at level 20% compare with M199 supplemented with (FCS) at level 10% ,and maintain the viability of gonads cell .

this result specifically dis agree with previous study reported that medium supplemented with 10% FCS was superior to medium supplemented with 20% FCS and stimulate the growth and proliferation of mantle tissue culture for *Mytilus galloprovincialis*(16) . in this work we think that ,the medium supplemented with 20% FCS prolonged the gonads cells still a live for more than two weeks and maintained the viability of these cells in these period , because FCS contains various plasma protein, polypeptide, fat, carbohydrate, growth factor, hormones and inorganic mineral, etc., all these substances keep the physiological balance of promoting cell growth and viability , also these substances Provide essential nutrients such as amino acids, vitamins, inorganic minerals, fat, and nucleic acid derivatives, which are essential nutrients for cell growth and maintain cells viability, these reasons supported by(17).also FCS Provide the basal media with hormone and various growth factors such as insulin, adrenocortical hormone (hydrocortisone, dexamethasone), steroid hormone (estradiol, testosterone, and progesterone), etc. (5).

The growth factors that contained in FCS include fibroblast growth factor (FGF), epidermal growth factor (EGF), pleteletdericed growth factor (PDGF),provide binding protein(s) For example, the albumin carries vitamins, fat (fatty acid, cholesterol) and hormones ,while transferrin carries iron ,provide protection for some specific cells Some cells (such as epithelial cells, myeloid cells) can release protease, which can be neutralized by the anti-protease ingredient in the serum(18).

FCS is widely used to terminate the effect of the trypsin. Serum albumin facilitates the serum viscosity and protects the cell from mechanical damage, especially in the suspension cell culture. The trace elements and ions, such as SeO3 and Selenium, play very important role in metabolic detoxification (19).

All these factors that mentioned above supported our assumptions that the basal medium supplemented with FCS at level 20% will be stimulate the growth and maintain the viability of gonads cell for *bellamya bengalensis in vitro.*

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