Detection the effect of culture medium supplied with fetal bovine serum on the proliferation of gonad cell cultured for rats Rattus norvegicus

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Abstract. This paper aimed to evaluate the role of fetal bovine serum (FBS) on the proliferation cultured gonads cells of white male rats by detection the impact of suitable concentration of (FBS) for gonads cell cultured in vitro. To perform the experiment the gonads of the rats were dissection out to obtain the gonad cells, then the obtained gonad cells were cultured in minimal essential medium (MEM medium) added to it different concentration of fetal bovine serum (10%,20%), the cells were cultured in (MEM medium) supported with (FBS) showed higher proliferation ratio (91.66±0.33) compare with the proliferation ratio at minimal essential medium (MEM medium) 76.66±0.33 and control (4.66±0.33), in the same direction the proliferation ration in medium supported with (20% FBS) 34±0 exceed the proliferation ratio in the medium supported with (10% FBS) 18±0 after two weeks from the culturing of gonad cells. FBS had positive role to support the proliferation of gonad cells for white rats male.

Key words: FBS, MEM medium, Rattus norvegicus, rats, in vitro

Introduction

The cell and tissue culture are using increasingly, and new in vitro supplements are progressed. The developed use of in vitro procedures in basic research and applied biomedical sciences increasing exponentially. Vertebrate cell and tissue culture has acquired important effect in essential and biomedical research (3) the effective proliferation and maintenance the viability of animal cells cultured in vitro, especially primary and secondary cell culture with culture conditions demand at least the optimal physiological parameters such as pH, temperature and oxygen supply in vivo (9).

The most culture media that are used in vertebrates cell and tissue culture must be supported and provided with all basic factors and ingredients for cell proliferation and maintain the optimal growth the essential bio factors are so necessary to cells cultured metabolism and supplied the metabolism energy for cells proliferation (11).

Also vitamins and trace elements have physiological and catalytic effects in long –term primary cells and and tissue culture, in the same direction the inorganics ions have important role in maintain
the media pH and osmolarity within acceptable physiological conditions that mimic the physiological and catalytic conditions in vivo (13).

The addition of animal serum to the basal culture media is necessary for cell proliferation and promotion cellular growth in vitro and for the stimulation of proliferation, serum that are used in cell and vertebrate tissue culture are obtained from adult or new born (17).

Generally serum as well as fetal bovine serum (FBS), is consist of a lot of ingredients in mixture form. These components have different physiological effects such as binding, and transport proteins and acceleration cell growth and cellular proliferation especially by hormonal stimulation and amino acids that promote cellular differentiation (8).

For all reasons that mentioned above, We hypothesized that the minimal essential medium (MEM) with tow concentration (10%, 20%) from fetal bovine serum (FBS) could be used to stimulate the cellular proliferation for white rat gonad cells and the ability of minimal essential medium (MEM) provided with 20% fetal bovine serum (FBS) to increase the proliferation ratio for longest possible period.

**Material and methods.**

In the current paper we used white rats male of the type Rattus norvegicus (weight ±200 gm.) the animals were carried to the lab 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 (4). The Gonads explant from the Rattus norvegicus 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 (4,15).

The cell suspension was transferred in a multi-wells and left at 15°C. Cells viability was checked every three days by using trypan blue dye (3), the culture medium was substituted tow times per week order to avoid possible contamination (4,15).

**Results and Discussion**

This paper proposed to detection the role of fetal bovine serum (FBS) added to minimal essential medium (MEM) on the proliferation of gonads cells, fetal bovine serum (FBS) are used at tow concentration of serum 10% and 20%.

The results of this paper reported that there was significant differences (p ≤ 0.05) between (MEM) provided with FBS and MEM medium at the first day, when the proliferation of gonad cells were (87.66±0.33) in 10% FBS added to MEM medium and (91.66±0.33) in 20% FBS added to MEM medium compare with (76.66±0.33) in MEM and (4.66±0.33) in control (table 1 and fig1).

This results showed that, there were significant differences (p ≤ 0.05) between the proliferation of gonad cells in MEM provided with 20% FBS, MEM provided with 10% FBS, MEM medium and control after three days from gonads culturing, when the ratios of proliferation were (83.66±0.33, 76.66±0.33, 70.66±0.33, 0±0) respectively.

After six days of gonads culturing, there were significant differences (p ≤ 0.05) between the cellular proliferation of gonads especially between the MEM medium supplied with 20% FBS when the
proliferation was (76±0) and the proliferation in MEM provided with 10% FBS, MEM medium and control when the proliferation ratio were (63.66±0.33, 61.66±0.33, 0±0) respectively.

Our results pointed out there were significant differences (p ≤ 0.05) between the proliferation of gonad cell in the all culture media At the ninth and twelfth days, when the proliferation were (52±0, 40±0, 23.66±0.33, 0±0) after nine days of gonads culturing.

And (34±0, 18±0, 0±0, 0±0) at the twelfth day from gonads culturing in MEM supplied with 20% FBS, MEM provided with 10% FBS, MEM medium and control respectively.

After fifteen days from gonads culturing there was clear significant differences (p ≤ 0.05) between the proliferation ratio of gonad cell in the all culture media, when the proliferation was 20±0 in MEM media provided with 20% FBS, while the viability was (0±0) in MEM supplemented with 10% FBS, MEM medium and control (table 1, figure 1).

Table 1: the ratio of proliferation for gonad cells that cultured in MEM and MEM media supplied with 10% and 20% FBS.

| Culture media       | 1 day     | 3 days    | 6 days    | 9 days    | 12 days   | 15 days   |
|---------------------|-----------|-----------|-----------|-----------|-----------|-----------|
| control             | 4.66±0.33 | 0±0       | 0±0       | 0±0       | 0±0       | 0±0       |
| MEM                 | 76.66±0.33| 70.66±0.33| 61.66±0.33| 23.66±0.33| 0±0       | 0±0       |
| MEM+10 FBS          | 87.66±0.33| 76.66±0.33| 63.66±0.33| 40±0      | 18±0      | 0±0       |
| MEM + 20 % FBS      | 91.66±0.33| 83.66±0.33| 76±0      | 52±0      | 34±0      | 20±0      |
| LSD                 |           |           |           |           |           | 4.845     |

Figure 1: comparison between the proliferation of gonad cells in MEM medium and MEM medium supplemented with different concentrations of FBS.

One of all animal sera, fetal bovine serum (FBS) was known to progress the in vitro cells culture of a lot of cell types including vertebrates, from the elementary level of the cell and tissue culture in vitro the media were supplied with fetal bovine serum (FBS) to improve proliferation of culturing cells and maintain the cells viability (10). Fetal bovine serum had the ability to expanse the growth of cultured cells due to might the (FBS had a lot of mediators and to growth factors, (12).
In our study, In order to enhance cell culture techniques, the MEM medium supported with low concentrations of FBS in experiments introduced to gonads cells cultured of male rats. This paper designed to estimate the effect of fetal bovine serum (FBS) in two different concentrations added to MEM medium on in vitro gonads cell cultured. The results of this paper agree with (13) who reported that basal culture media supplied with FBS promote the cell proliferation and growth in the cultured cells.

The results pointed out FBS at level 20% added to MEM medium had positive effect on cellular proliferation and viability, when the proliferation get the highest rates and the media supported with 20% FBS extended the viability of cultured cells for fifteen days approximately.

The results of our study also compatible with previous studies has been showed that the use of fetal bovine serum at concentrations of 10–20% has been reported to be serious effect for vertebrates cell cultures in vitro (14,16). the components of fetal bovine serum (FBS) such as (proteins, hormones, lipids and growth factors) promoted cell proliferation. When fetal bovine serum has been added to basal growth medium in this study (MEM) it has been demonstrated that and proliferations and maintain the viability of gonads cells, but our results dis compatible with (15) they reported that the fetal bovine serum (FBS) had less roles when use as supplements in mammalian cell cultures in vitro studies.

this result specifically dis agree with previous study reported that medium supplemented with 10% FBS was superior to medium supplemented with 20% FBS and stimulate the growth and proliferation of mantle tissue culture (4). in this paper we think that the medium supplemented with 20% FBS prolonged the gonads cells still alive for more than two weeks and maintained the viability of these cells in these period, because FBS 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 proliferation, 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 proliferation, these reasons supported by (17), also FBS provide the basal media with hormone and various growth factors such as insulin, adrenocortical hormone (hydrocortisone, dexamethasone), steroid hormone (estradiol, testosterone, and progesterone), etc. (4).

it was well reported in the present study that the increase in fetal bovine (FBS) level from 10% to 20% had positive effect on cells growth and maintain cells viability, but the most effect was MEM supplied with (FBS) at level 20% compare with MEM supplemented with (FBS) at level 10% and maintain the viability of gonads cell cultured.

The growth factors that contained in FBS include fibroblast growth factor (FGF), epidermal growth factor (EGF), pleteledericed 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 (17).

FBS 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 (11).

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