CHICKEN EMBRYO FIBROBLAST CULTURE AS AN *IN VITRO* MODEL FOR ANIMAL SCIENCE RESEARCH

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ABSTRACT

Cell culture systems offer potential alternate platform for *invivo* or living models so that extensive researches are possible in virus propagation, physiological changes and for vaccine development. Chicken embryo fibroblast (CEF) culture is a primary type of cell culture which has much application in poultry virus as well as physiological researches. This technical article explains the procedure for successfully establishing a CEF culture system in laboratory along with images.

INTRODUCTION

Cell culture systems are established in the laboratory when cells of animal origin are cultured at sterile *in vitro* environment with appropriate and supplemented media. This has been followed from 19th century onwards and used mainly for isolation and propagation of viruses which can survive and multiply only in a living system. Subsequently, Based on the tissue of origin and nature of cell multiplication, cell cultures are basically of three types: **primary culture** (freshly isolated cell from animal tissue, heterogeneous and slow growing in nature), **secondary culture** (cells isolated from primary culture, modified for longer life and multiplication) and **cell lines** (permanent cells which can be propagated continuously and indefinitely). Chicken embryo fibroblast culture is one of the primary cell culture system prepared from embryonated chicken of 9-11 days old. At this stage of chicken embryo, fibroblast cells are the predominant one in the muscle tissues which will be collected aseptically and treated to form single cell suspension. Successfully established fibroblast culture can be used for propagation of viruses especially of Herpes viridae family (Marek's disease virus, Duck Plague virus etc.) and as an *in vitro* model for exploring various physiological responses.

REQUIREMENTS

Aseptic environment is mandatory for establishing a successful cell culture system in the laboratory. Handling of the tissues and cell preparation are to be done only inside a biosafety cabinet. All the reagents, glasswares and plasticwares used are to be ensured for sterility. Reagents include Hank's Balanced Salt Solution (HBSS), Antibiotic-antimycotic solution, 0.25% Trypsin, growth medium (DMEM) with 10% serum Foetal bovine Serum (FBS).

PROCEDURE

Chicken embryo fibroblast culture is established from aseptically collected chicken embryo (of 9-11 days old) following the standard protocol. Briefly, egg sterilized with 70% alcohol is kept upright with air space on top, break the egg at air space, and embryo is carefully taken out into sterile petri plate containing HBSS. After slight washing in HBSS, head, four appendages and viscera are carefully removed and skin is peeled off. Muscle part containing fibroblast is put into another petri plate, washed twice with HBSS and cut into pieces. Tissue is added with trypsin (0.25%)-HBSS mixture and kept on magnetic stirrer for 10 min at spinning rate of 400-500 for trypsinization. Mixture will be strained into sterile beaker to obtain single cell suspension and supplemented with growth media (preferably DMEM containing 10% serum and 1xantibiotic-antimycotic solution). Cells will be pelleted by centrifugation (1000rpm for 5-10 min), finally collected cells will be resuspended in 3-5ml growth media. After assessing the cell concentration, additional growth medium can be added to make up the required cell concentration (1-2x10^6 cells per ml concentration) and cells can be cultured in tissue culture flask or plates at 37◦C in a humidified chamber with 5% CO2 level. About 6ml cells are added in each 25cm^2 culture flask while 1ml cells are added per well in 12-well plate. Once the cells attain 90% confluency by 24-48 hrs incubation, media need to be changed and cells can be infected with virus for cultivation or titration. Media change is needed at every alternate day. Virus induced microscopic lesions in the cells (in the form of plaques) will be evident from 3-4 days onwards and enumerated by 6 day.

APPLICATIONS

Successful establishment of cell cultures for the vaccine research at both human and veterinary sectors paved way for the development of various vaccines that include hepatitis vaccine, rabies vaccine, flu vaccines, etc. for human use and FMD vaccine, Rinderpest vaccine, PPR vaccine, Goatpox vaccine and recently Classical Swine fever vaccine for veterinary use. Chicken embryo fibroblast culture is mainly used for the production of Marek's disease (MD) virus vaccine production since the virus is propagated and titrated in this culture system. MD produce plaques in the fibroblast after 4-5days incubation and these

plaques are counted for assessing the titre of the virus in terms of plaque forming units (PFU). CEF cultures are also been explored for bioenergetics, molecular events and cellular events involved in muscle tissue proliferation and differentiation.

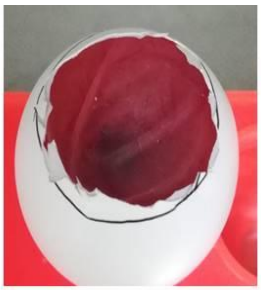

Fig 1: 10-day old embryonated chicken egg - opened at air space

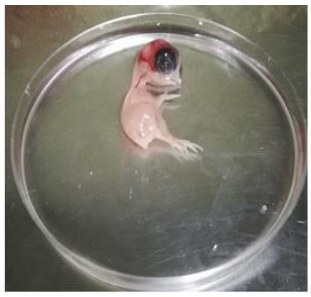

Fig 2: 10-day old chicken embryo

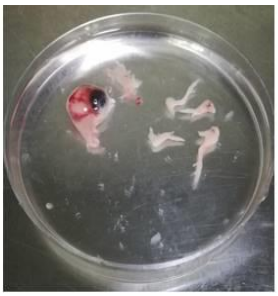

Fig 3: 10-day old chicken embryo-with parts removed

Fig 4: muscle pieces collected from 10-day old chicken embryo for fibroblast culture

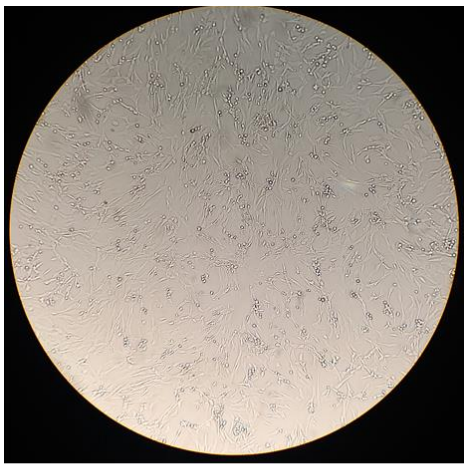

Fig 5: Chicken embryo fibroblasts after 24 hrs incubation

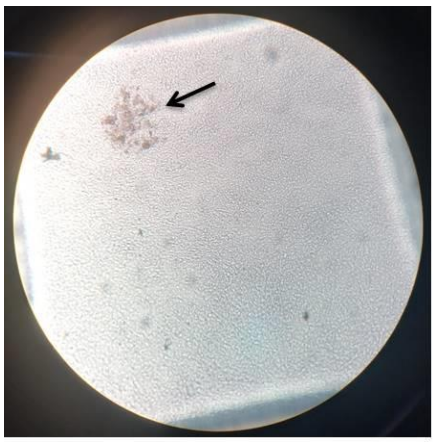

Fig 6 : Plaques formed in chicken embryo fibroblasts culture after 4days of MDV infection

CONCLUSION

Successful establishment of cell cultures for the vaccine research at both human and veterinary sectors paved way for the development of various vaccines that include hepatitis vaccine, rabies vaccine, flu vaccines, etc. for human use and FMD vaccine, Rinderpest vaccine, PPR vaccine, Goatpox vaccine and recently Classical Swine fever vaccine for veterinary use. CEF offers a much easier platform for various researches in the field of vaccine development, immunological and physiological changes etc. especially in poultry.

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