# GOAT MILK WHEY AS A FOETAL CALF SERUM SUBSTITUTE FOR THE GROWTH OF CHICKEN EMBRYO FIBROBLASTS

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## ABSTRACT

Hank's balanced salt solution (HBSS) was supplemented with 5, 10, 15, 20 and 25% concentration of goat milk whey for the growth of chicken embryo fibroblasts. The tissue culture flasks in which media was supplemented with 25% concentration of goat milk whey supported the growth of chicken embryo fibroblasts. That growth was comparable to the growth observed in tissue culture flasks having 5 and 10% of foetal calf serum. No growth was observed in which foetal calf serum or goat milk whey was not supplemented in the medium.

## INTRODUCTION

Milk is a nutritious source of high quality proteins, possessing good functional properties, due to its unique amino acid sequence and many desirable physicochemical attributes (Lincoln, 1989). For the purpose of analysis and isolation of the components, milk proteins are fractionated in to three classes i.e., casein present in micelles, whey proteins in solution and fat globule membrane proteins on the surface of fat globules. When casein is precipitated from milk by rennin, the resulting whey contains the soluble proteins including the proteose-peptone fragments and casein macropeptide from the splitting of β-casein. Milk whey contains a heterogeneous group of proteins that are either derived from blood or synthesized in the mammary glands. Some proteins are common to blood and milk but three major components of milk proteins i.e., casein, α-lactalbumin and β-lactoglobulin are synthesized exclusively by the mammary glands (Larson, 1985).

Cell culturing is now widely used in diagnostic laboratories for isolation, identification and propagation of virus. The crucial role of suitable cell culture media and its economic aspects are well known. Among the biological fluids that proved successful for culturing cells outside the body, serum has gained the most wide spread usage. Serum is an extremely complex mixture of balanced growth promoting and growth inhibiting activities (Freshney, 1986). The present praper reports the use of varying concentration of goat milk whey as a substitute to foetal calf serum for *in vitro* propagation of chicken embryo fibroblasts.

## MATERIALS AND METHODS

Ten fresh goat milk samples were collected. The milk was centrifuged at 3,000 rpm for 20-25 minutes. The condensed fat in the form of superficial layer was removed with a glass rod. Casein was separated from the fat free milk by coagulation with rennin (Rennet-Difco). For each ml. of fat free milk, 0.01 ml of 2% rennin solution was added and mixed. It was incubated at 37°C till curdling. The curd was broken with a glass rod and the whey was separated from clotted milk by centrifugation at 2,000 rpm for 20 minutes. The whey thus separated was filtered through Seitz filter (47mm diameter) and then subsequently through membrane filter of 0.22 μm pore size (Akhtar, 1990). The purified whey obtained in this whey was subjected to cell culture studies.

### Chicken Embryo Fibroblast Culture

Nine to eleven days old embryonated hen's egg was candeled to test its viability. The egg shell was sponged with methyl alcohol. Egg shell was cut just below the air sac and opened. The embryo was lifted carefully with a sterilized foreceps in to a sterilized petri plate having phosphate buffered saline (PBS). The head and appendages were removed with scissors. The breast and high muscles were removed and washed in phosphate buffered saline containing 1000μg of streptomycin and 1000 IU of penicillin/ml. The tissues were transferred in 20 ml of PBS containing trypsin (E-Merck) at the rate of

(3-4mm) and subjected to continuous gentle stirring at 37°C for one hour on magnetic stirrer. Then the beaker containing cells was immediately put in ice box. The cell suspension was filtered through sterilized muslin cloth into a sterilized centrifuge tube. The cells were centrifuged at 3,000 rpm for 15 minutes. Trypsin solution in the form of supernatant was discarded. The sedimented cells were washed in 10 ml of PBS. The washed cells were resuspended in 1/2 ml of filtered Hanks' balanced salt solution (HBSS). Tissue culture flasks of 25 ml were incubated with 0.1 ml cell suspension containing media as mentioned in Table 1.

Tissue culture flasks were incubated at 39 ± 1°C overnight. The attachment of chicken embryo fibroblasts was observed after 2 hours of incubation under inverted microscope. Growth of chicken embryo fibroblasts was observed after 24 hours of incubation under inverted microscope.

## RESULTS AND DISCUSSION

After two hours of incubation there was attachment of chicken embryo fibroblasts. Growth of chicken embryo fibroblasts was observed after 24 hours of incubation. The results are presented in Table 1. 25 % goat milk whey in the growth medium is a potent stimulator for the growth of chicken embryo fibroblasts in culture. Cells in the medium supplemented with low concentrations (5, 10, 15 and 20%) of goat milk whey failed to divide, remained viable but sparse. Growth of chicken embryo fibroblasts was observed when the medium was supplemented with 5 and 10% of foetal calf serum. In the absence of milk whey or foetal calf serum, growth was not observed in the tissue culture flasks.

Successful specific formulations for large scale cell culture production of recombinant proteins tends to be well kept industrial secrets. Published work on the subject is scarce, what is evident as the scale of rDNA cell culture increases, however, is a rising demand for workable serum free media.

Hank's balanced salt solution which was supplemented with low concentrations (5, 10, 15, 20%) of goat milk whey did not support the growth of chicken embryo fibroblasts, cells remained viable but unable to divide may be due to low concentration of proteins. This observation was supported by Wasley and May (1970) that low concentrations of foetal calf serum could be used to maintain medium but this did not support the growth of cells. Tissue culture flasks supplemented with 25% goat milk whey supported the growth of chicken embryo fibroblasts. Growth medium supplemented with 5 and 10% foetal calf serum supported the growth of chicken

Table: 1 Different concentrations or goat milk whey and foetal calf serum used for the growth of chicken embryo fibroblasts.

| Tissue culture flask | HBSS (ml) | Goat milk whey Volume (ml) | % | Foetal calf serum Volume (ml) | % | Growth of CEF* |
|---|---|---|---|---|---|---|
| 1 | 15 | 0.75 | 5 | - | - | ± |
| 2 | 15 | 1.5 | 10 | - | - | ± |
| 3 | 15 | 2.25 | 15 | - | - | ± |
| 4 | 15 | 3.0 | 20 | - | - | ± |
| 5 | 15 | 3.75 | 25 | - | - | + |
| 6 | 15 | - | - | 0.75 | 5 | + |
| 7 | 15 | - | - | 1.5 | 10 | + |
| 8 | 15 | - | - | - | - | - |

* Chicken embryo fibroblasts

embryo fibroblasts. This is correlated with the findings of Hitchner *et al.* (1975) that 5 and 10% foetal calf serum supported the growth of cells. Watson *et al.* (1992) purified bovine colostral whey by cation exchange and reverse-phase chromatography and reported the stimulatory effect on the growth of L6 rat myoblast, Balb/c 3T3 mouse fibroblasts and BHK-21 cells with equal or greater potency than foetal bovine serum.

Fassolitis *et al.* (1981) substituted the foetal calf serum with non fat dry milk filtrate. The non fat dry milk supplemented medium supported the growth of all epithelial cells tested, but 2 fibroblast type cultures failed to replicate. According to the findings of Tolar *et al.* (1983), defined medium supplemented with growth promoting alpha globulin supported the formation, differentiation and enervation of myotubes in cell culture from chicken embryo. Tozzini and Bandecchi (1985) compared the growth medium containing foetal bovine serum (MEM-FBS) with medium containing growth factors and nutrients (MES-N). No differences were observed between the two media, either in the growth of PK1-3, VERO and IMR-31 cells, or in the virus titres obtained when they were inoculated with vesicular stomatitis virus. Ashfaque *et al.* (1994) observed that 10 % buffalo colostrum whey supported the growth of chicken embryo fibroblasts hence could be used as a substitute to foetal calf serum because the proteins of the colostrum whey had a comparable electrophoretic pattern to that of foetal calf serum. Brown and Blakeley (1983) have also reported that mammary secretions from goats, sheep and cows at concentrations as low as 0.5% (v/v) stimulated the proliferation of mouse 3T3 fibroblasts in culture. The levels of cell growth promoting activity were at least 25 fold higher in precolostrum and colostrum than in mature milk.

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