EXPERIMENT NO: 1
DATE : **20-21 March 2018**

PREPARATION OF MEDIA (MEM + 10% FBS)

AIM : To prepare desired medium for the given Animal cell culture.

PRINCIPLE: All the Animal cells can be grown in a liquid culture medium consisting of a mixture of vitamins, salts, glucose, amino acids and growth factors. Moreover, foetal bovine serum is an easily available source of growth and attachment factors. Antibiotics are added to prevent the growth of bacteria. Under these conditions cells will grow at physiological pH (7.4) and at body temperature (37ºC) to form a monolayer on the culture vessels.

MATERIALS REQUIRED
Medium (MEM) contain gentamicin
Foetal bovine serum - FBS
Membrane filter (Millipore 0.2μm)
15 ml tube
filter
Syringe

METHOD:
Sterilise the laminar/bench by 70% ethanol.
1. Prepare 10 ml of medium containing 10% foetal bovine serum (FBS) - take 9ml of MEM add 1ml of FBS
2. Filter sterilise using 0,2 μm filter.
3. Warm up medium in water batch for at least 20min.

EXPERIMENT NO: 2
DATE : 20-21 **March 2018**

PRIMARY CELL CULTURE

Aim : To perform primary cell culture technique using sheep foetuses or sheep adult tissue under aseptic condition

Introduction: Development of techniques for the *in vitro* culture of animal cells has proven valuable for the study of structure and function of cells under controlled conditions. Further, cultured cells find important applications in vaccine production, hybridoma production and in chromosome karyotyping. Almost any tissue can be cultured, if it is appropriately dispersed.
Cultures started fresh from tissues are called **primary cultures.** A method for the propagation of primary cultures of sheep cells is given below which can be adopted for the culture of other embryonic tissues derived from different species. Often primary and secondary cultures derived from normal tissue have finite life span similar to their *in vitro* life. However, some cells out of a large population are secondary cultures by pass this definite life span and get immortalized with a capacity to divide indefinitely and these are called cell lines. Many cancer cells have a capability to divide indefinitely in culture. Normal cells transformed by viruses and chemical carcinogens also become continues cell lines.

Principle
Primary cultures are usually prepared from large tissue masses. Thus, these cultures contains a variety of differentiated cells. Embryonic tissues are preferred for primary cultures due to that the embryonic cells can be disaggregated easily and yield more viable cells. The quantity of cells used in the primary culture should be higher since their survival rate is substantially lower.

Materials and reagents Required you need

Materials:
Culture dish (non treated) - 2x
Culture dish (treated for cell culture) - 1x
1 pairs of scissors
1 needle
1 blade
15 ml tube - 3x (1 for MEM+10%FBS, 1 for PBS, 1 for tissue fragments)
1 eppendorf (for trypsin)
5-6 or more pasture pipette
Wast container

Reagents:
1 ml of Trypsin - (transfer into 1.5ml eppendorf and warm up in 37°C water batch)
10 ml of MEM medium + 10% FBS - prepared before - warm up in 37°C water batch
10 ml of PBS - Calcium, Magnesium free – phosphate buffered saline (PBS) - 10 ml (warm up in 37°C water batch)

Tissue from sheep foetus or adult tissue (small pice of tail or ear or skin).

Methods
1. Put on gloves and lab coat.
2. Sterilise/clean the laminar/bench by 70% ethanol.
3. Collect all materials/reagents required for experiment and prepare work place.

4. Isolate small piece (0.5 - 1cm) of tissue or adult tissue from sheep/mouse using scissors and/or blades.
5. Transfer tissue in culture dish (non treated) and wash with 1ml of PBS.
6. Remove hair, fat or death cells if you isolate piece of tissue from adult organs (ex. ear)
7. Transfer the tissue to the drop of trypsin (cover of non treated dish).
8. Cut tissue into small 1-2 mm pieces (keeping always in trypsin) (mechanical and enzymatic desegregation). Using needle, blade or scissors (as you prefer).
9. At the end of the above period add 5ml of medium containing serum and stir the contents for 1-2 more minutes to inactivate the action of trypsin.
10. Collect and transfer all the tissue/ trypsin/medium solution into a 15 ml tube (using Pasteur pipette).
11. Centrifuge at ~ 1000 rpm for 5mins.
12. Pour out the supernatant and resuspend the pellet in 5ml of warm PBS.
13. Centrifuge at ~ 1000 rpm for 5mins.
14. Pour out the supernatant and resuspend the pellet in 1ml of medium (MEM + 10% FBS).
15. Distribute all tissue piece equally to all the culture culture bottles and incubate at 37ºC + 5% CO2.
16. Observe culture every day under the microscope
17. Change medium after 2-3 days of culture (Fig. 1).

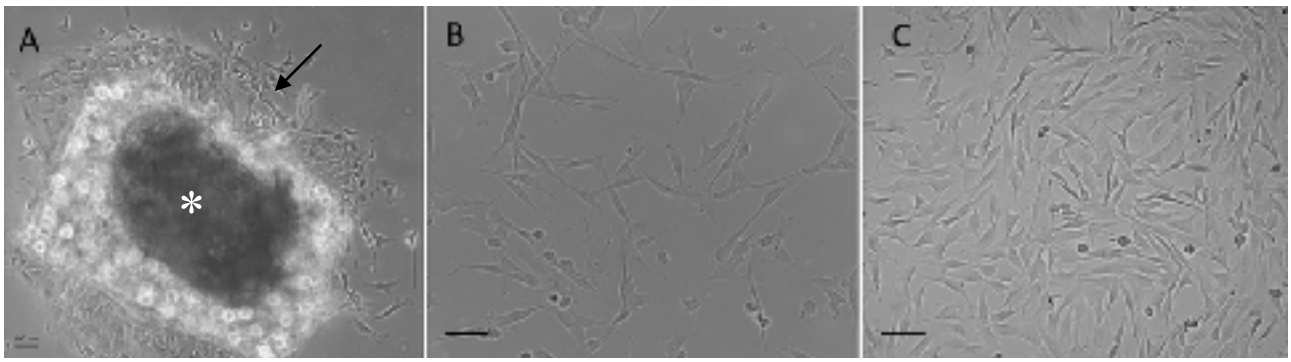

Figure. 1. Sheep primary cell line isolated from 2 moths foetus after 2 day (A), 5 (B) and 7 days in culture. * tissue fragment, arrow fibroblasts.