Testing the Milk of Cow and Goat as an Alternative for Fetal Bovine Serum in Cell Cultures

Cell cultures are essential in the field of biology as they are used in many forms of research such as understanding the behaviors of wild type cells and diseased cells. Tissue engineering and cellular agriculture are also two fields of study where the focus is cell cultures. The process of growing cells requires a growth media which contains the required nutrients to enable the cells to proliferate. Currently, the most prevalent growth media used in the scientific community is Fetal Bovine Serum (FBS), which is derived from the blood of an unborn calf (Lee et al., 2022). Currently, the only legal way to get blood from an unborn calf is from accidental slaughter of pregnant cows. This both has ethical and sustainability concerns. On the sustainability aspect, since FBS can only be sourced from accidental deaths meaning the supply of FBS is not always constant. This causes a frequent fluctuation of the price for FBS and sometimes makes it difficult to obtain it. Additionally, due to its high cost, FBS hinders researchers with a lower budget from carrying out research hence most advancements in biology are likely to occur in well developed countries. Secondly, it is highly unethical to kill pregnant cows as it is vital to consider the welfare of animals and treat them with respect and compassion.

Process of manufacturing FBS

There are six major steps in the production of FBS. The first step is the slaughter of a pregnant cow without knowledge. Upon the realization of the fetus, the fetus is sterilized in a sterile environment and blood is extracted via a needle. This step can only be proceeded in government approved slaughterhouses to prevent uncontrolled slaughter of the fetus. The collected blood is refrigerated to induce coagulation. When the blood is coagulated, the serum gets separated when centrifuged. This removes clotting factors and cellular

components from the blood. After this process, we are left with a highly pure serum with an orange hue. Then the serum is filtered through 0.1 µm triple filtration, a filtration chain and is sometimes treated with irradiation (using gamma rays) (Lee et al., 2022). Finally, the serum is stored in a sterile packaging and frozen until further use. This serum can be stored for an average of 5 years (Muniaraj et al., 2007).

Why is FBS Hard to Replace

Firstly, the chemical makeup of FBS is not fully discovered yet, meaning it is not possible to make an exact replica of FBS from scratch. Additionally, some of the components in FBS such as (examples) are very expensive to reproduce and cannot be mass produced. Even if some viable replacements are available, researchers are more likely to use FBS as it is widely accepted by the scientific community (Lee et al., 2022). Using FBS also results in the best cell proliferation therefore giving more cells to work with to carry on the research. Having more cells decreases cell to cell variability when looking at all the cells.

Why is milk a Viable alternative?

First, milk is an animal product just like FBS therefore, this is naturally more protein content, which is an important factor in cell proliferation. Milk also has other important factors that contribute to cell growth such as albumin which are hard to find in other compounds. During the first few months in a mammal's life, milk is vital for its growth. This means milk has components necessary for growth of life and that's what cell cultures are, life (Foroutan et al., 2019).

In a recent study done by Muniaraj et al.(2006), It was shown that the use of goat milk with the addition of Media 199(elaborate) is more effective than FBS in culturing *Leishmania donovani* Promastigotes by more than 40%. The high content of protein, glucose, calcium, and triglycerides is hypothesized to be the cause of such growth (Muniaraj et al., 2006).

Problem Statement

Fetal Bovine Serum is a widely used growth media for cell cultures but has an unstable production process and is very expensive which hinders people from underprivileged areas to perform research.

Objective

This Project will investigate the effect of using cow's and goat's milk as an alternative for FBS. This will be achieved by culturing multiple cell lines using both FBS and the milks (cow and goat) and comparing the cell growth and quality.

Obj. 1: Sterilize the Milk and colostrum and append additives such as DMEM and antibiotics to make a complete growth medium.

Obj. 2a: Grow the cell line over a period of 2 weeks with both the milk-based mediums, and FBS.

Obj. 2b: Using the data from 2a to refine the method and experiment with different concentrations of FBS and the milk-based medium.

Hypothesis

Hyp. 1a: It is hypothesized that the average growth of cells will be less in the bovine milk-based media compared to the FBS based media.

Hyp. 1b: It is hypothesized that the average growth of cells will be the same in the goat milk-based media compared to the FBS based media.

Hyp. 2a: The average cell growth of the colostrum-based medium will be higher than the FBS media.

Competitor Analysis

Currently in the market, there are many FBS alternatives such as FBM™, TesR™, Essential 8™, XerumFree™, Lipogro™, and okara extract. The problem with most of the alternatives listed above is that it is hard to produce therefore the cost is still an issue. On top of that, they are not as effective (Kolkmann et al., 2019). Although okara is cheap and easily obtainable, it still performs at a much lower level than FBS (Teng et al., 2023). According to Figure 1, the trademarked growth mediums above peak its performance 30% of FBS's average performance in growing primary myoblast cells. The problem with FBS is that the chemical composition of FBS is not fully defined therefore any artificial serum trying to replicate FBS exactly will have a lower success rate.