Procedure

Equipment and Materials

Once taken from the fridge, the colostrum and milk were rested in water until fully thawed then transferred into 50 ml conical tubes. Then they underwent centrifugation at 870 RCF for 20 minutes. Then, the fat that was floating on the top was removed using metal spoons and placed into another container. The milk and colostrum again were centrifuged at 470 RCF for 5 minutes to get any excess fat. Again, the fat was collected and this time the milk and colostrum were transferred to different tubes as the pellet was not needed. The milk and colostrum were placed in 60 degree water for 30 minutes to eliminate some of the bacteria. The milk was then rested at room temperature until the milk was at most 30 degrees Celsius. The milk was stored in a fridge at 2-4 degrees Celsius.

After the Tyndallization process, the milk was transferred into new tubes and placed in a centrifuge for 30 minutes at 3,000 RCF. Then, the fat floating above was aspirated. The milk was transferred into new tubes and the cell pellet that formed from the centrifugation was disposed of. The milk and colostrum were run through 0.45-micron filtration to ensure further sterility.

To check the sterility of the milk, the 1 ml of the milk was diluted with 9 ml DMEM and was placed in an agar plate. The same was done with colostrum. The two plates were incubated at 37 deg for 30 hours along with another agar plate with only DMEM as a control. After the 24-hour period, the agar plates were put under a microscope to examine for contamination but none was observed

To create the growth medium, 0.1% glutamax, 0.1% of penstrep and 95% DMEM was added to 5% milk, colostrum or FBS.

In the culture plate with 3T3 cells provided by Dr. Ambady, the culture medium was aspirated using a pasteur pipette. The cells were rinsed twice with 5 ml of DPBS (-) and aspirated again. Subsequently, 3 ml of 0.25% Trypsin-EDTA solution was added, and the cells were incubated for 5-10 minutes at 37 degrees Celsius in a 5% CO2 environment. Following the incubation, 7 ml of FBS-based medium should be added to the culture to make a total volume of 10 ml. The cells were dispersed repeatedly by pipetting, and the cell suspension was transferred into a 15 ml conical tube. A sample of 7 microliters was taken and saved for cell count. The rest of the cells are centrifuged from 200-250 RCF for 10 minutes.

Techniques

Tydallization was used in this project to eliminate the bacteria and other microorganisms which might disrupt cell growth while still preserving the structure of the proteins that exist in the milk. Centrifugation was used to eliminate the fat globules which disrupt the view of cells when looked under the microscope. A regular protocol provided by Dr. Ambady was used to culture 3T3 cells. The only modifications made to the protocols was replacing FBS with the milk and colostrum alternatives.