Project Notes:

Project Title: Testing the Milk and Colostrum of Goat as an Alternative for Fetal Bovine Serum In Cell Cultures

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Knowledge Gaps:

This list provides a brief overview of the major knowledge gaps for this project, how they were resolved and where to find the information.

Knowledge Gap	Resolved By	Information is located	Date resolved
What are the components in FBS	Reading article	Article 7	10/11/23
How to culture cells	Talking to professors and reading articles	Article 12 and logbook	11/15/23 and 12/2/23
Bovine colostrum and its use	Reading articles	Articles 12 13 and 14	12/8/23
Cellular assay	Reading articles and talking to professor	Article 18	12/2/23

Literature Search Parameters:

These searches were performed between (Start Date of reading) and XX/XX/2019.
List of keywords and databases used during this project.

Database/search engine	Keywords	Summary of search
Scopus	“Fetal Bovine serum”	I got the chemical composition of FBS, how it is made and general research articles on how FBS is useful.
Scopus	“Serum Free Media”	I got a lot of artificially made serum replacements and they were usually targeted towards one group of cells. They were overly specialized but there were some more general ones.
Science Direct	“ Natural Serum free growth media”	I saw articles on okara and other mediums based on waste products, they usually had minimal success rates.
Google Scholar	“Milk” and “FBS substitute”	There were very few articles regarding milk but had a lot of FBS substitute articles. I found a couple but they were old articles.
Google Scholar	“Bovine Colostrum”	Found more articles about milk and a lot based on mouse hybridoma cells. Most of the articles were old.

Tags:

Tag Name	
#Background	#methods

#analysis	#conclusion
#brainstorming	#ideas

Article #0 Notes: Title

Article notes should be on separate sheets

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Source Title	
Source citation (APA Format)	
Original URL	
Source type	
Keywords	
#Tags	
Summary of key points + notes (include methodology)	
Research Question/Problem/ Need	
Important Figures	
VOCAB: (w/definition)	
Cited references to follow up on	
Follow up Questions	

Article #1 Notes: Underground Cells Make 'Dark Oxygen' Without Light

Source Title	Underground Cells Make 'Dark Oxygen' Without Light
Source citation (APA Format)	Bolakhe, S. (2023, November 8). *Underground cells make 'Dark oxygen' without light*. Quanta Magazine. Retrieved December 15, 2023, from https://www.quantamagazine.org/underground-cells-make-dark-oxygen-without-light-20230717/
Original URL	https://www.quantamagazine.org/underground-cells-make-dark-oxygen-without-light-20230717/
Source type	Journal article/website
Keywords	Dark Oxygen, subterranean ecosystems
#Tags	#brainstorming
Summary of key points + notes (include methodology)	Scientists previously thought subterranean ecosystems had few organisms and were mostly anaerobic, but a recent study led by microbiologist Emil Ruff in Alberta, Canada, revealed that older groundwater samples contained more aerobic microbes than expected, challenging previous assumptions. This discovery, including the unexpected conversion of nitrites into nitrogen and oxygen gas by some microbes, has significant implications for understanding life in oxygen-deficient environments on other celestial bodies.
Research Question/Problem/ Need	How do aerobic organisms survive in subterranean ecosystems?
Important Figures	
VOCAB: (w/definition)	Methanogenic-a methane-producing bacterium, especially an archaean which reduces carbon dioxide to methane. dismutation-a process of simultaneous oxidation and reduction. used especially of compounds taking part in biological processes.
Cited references to follow up on	
Follow up Questions	1. Dark Oxygen: How do underground cells produce 'dark oxygen,' and what applications or implications does it have?

	2. Gold in Sewage Sludge: What methods are proposed for extracting gold from sewage sludge, and how economically viable is this process? 3. Cultured Meat Research: In what ways does fetal bovine serum contribute to the development of cultured meat, and are there ethical considerations in its use?

Notes:

- Initially, attempts to explain dissolved oxygen in samples as mishandling failed.
- Consistent dissolved oxygen content across samples hinted at a different source.
- Researchers questioned the foundation of subsurface ecosystems understanding.
- Groundwater oxygen is traced to microbes using mass spectrometry.
- Genomic sequencing identified a bacteria species producing oxygen through dismutation.
- Dismutation, previously considered rare, found to support aerobic microbe communities.
- Discovery reshapes knowledge of the subterranean biosphere and global compound cycles.
- Insight crucial for understanding extraterrestrial environments and potential life.
- Findings challenge preconceived notions about life's necessities.

Article #2 Notes: Sewage sludge could contain millions of dollars worth of gold

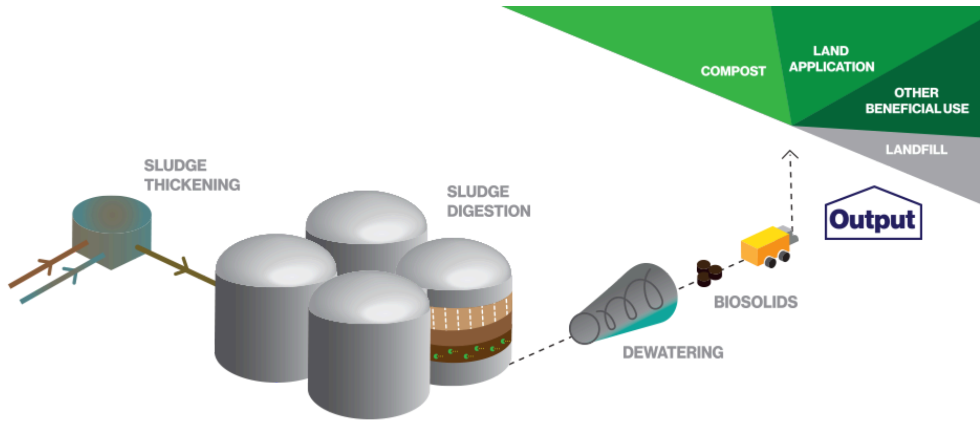
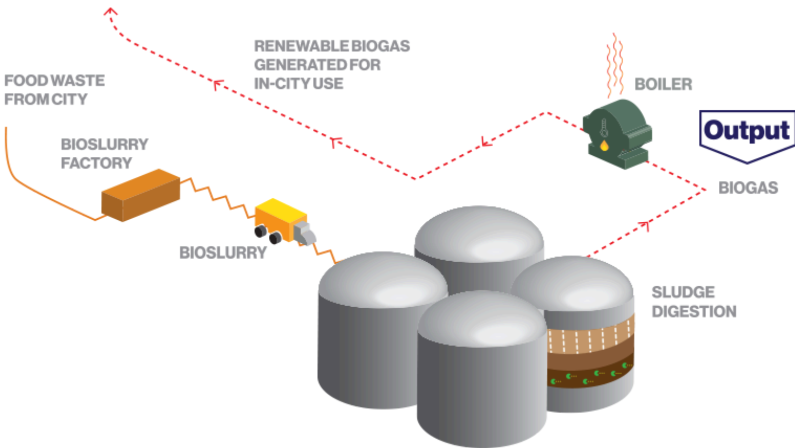
Source Title	Sewage sludge could contain millions of dollars worth of gold
Source citation (APA Format)	Cornwall, W. (2015, January 16). *Sewage sludge could contain millions of dollars worth of gold*. Science. https://www.science.org/content/article/sewage-sludge-could-contain-millions-dollars-worth-gold
Original URL	https://www.science.org/content/article/sewage-sludge-could-contain-millions-dollars-worth-gold
Source type	website/journal article
Keywords	sewage , sludge, bioplastics, fertilizer
#Tags	#brainstorming
Summary of key points + notes (include methodology)	Researchers at Arizona State University (ASU) have revealed the untapped value of sewage sludge, a byproduct of sewage treatment, containing valuable metals like gold, silver, platinum, copper, iron, and zinc. Advanced technology estimates that a city with a million residents could extract up to $13 million worth of these metals annually from sewage sludge, notably $2.6 million from gold and silver alone. This discovery challenges conventional waste perception, offering an innovative path for waste management and sustainability, albeit with challenges in efficient extraction and economic viability assessment.
Research Question/Problem/ Need	What is being lost in sewage treatment?
Important Figures	
VOCAB: (w/definition)	electroplating- the process of using electrodeposition to coat an object in a layer of metal(s)
Cited references to follow up on	https://pubs.acs.org/doi/full/10.1021/es505329q
Follow up Questions	1. How can we extract these materials? 2. How might the extraction of valuable metals from sewage sludge impact environmental pollution, considering the potential economic benefits? 3. Are there any existing technologies or methods being explored to make the extraction of metals from sewage sludge more economically viable and technically feasible?

Notes:

- Sewage sludge, a byproduct of wastewater treatment, may contain metals worth millions, including $2.6 million in gold and silver, according to a study from Arizona State University.
- The study suggests that cities could find value in sewage, turning it from a disposal problem to a potential resource.
- Extracting these metals from sludge faces economic and technical challenges, but it presents an opportunity for cities seeking alternative waste management solutions.
- Metals, including gold and silver, concentrate in sewage sludge due to a mix of toilet water, industrial effluent, and storm runoff.
- Sewage utilities currently struggle with toxic metals in wastewater, but the study estimates that a million-person city's sludge could contain up to $13 million worth of metals annually.
- While one Japanese city successfully extracted gold from sludge, it's not a common practice in the United States.
- About 60% of U.S. sewage sludge is used as fertilizer, raising concerns about contamination risks, while the rest is burned or dumped in landfills.
- The study contributes to the growing idea of viewing sewage as a valuable commodity, with other efforts involving phosphorus and nitrogen extraction and bioplastics production.
- Precious metals in sewage may originate from various sources like mining, electroplating, electronics, jewelry manufacturing, and industrial processes.

Article #3 Notes: Wastewater Treatment Process

Source Title	*Wastewater Treatment Process*
Source citation (APA Format)	*Wastewater Treatment Process - DEP*. (n.d.). Www.nyc.gov. https://www.nyc.gov/site/dep/water/wastewater-treatment-process.page
Original URL	https://www.nyc.gov/site/dep/water/wastewater-treatment-process.page
Source type	Government article
Keywords	Sewage, Wastewater Treatment
#Tags	#brainstorming
Summary of key points + notes (include methodology)	There are four steps in treating wastewater: preliminary stage where trash and debris such as bags are caught by a net and sent dumped in landfills, primary treatment where most of the sludge is removed, the secondary treatment where air is added for microorganism to consume small particles, and lastly disinfection where sodium hypochlorite is added to the water to disinfect it. The sludge that was removed in the process thickened and processed my microorganisms to create biosolids that can be used for farming. This process also releases methane which is used as energy to power the process.
Research Question/Problem/ Need	What is the process of wastewater Treatment?

Important Figures	 Shows Sludge Digestion is a static process and takes a long time. Also shows a wide range of areas where biosolids are deposited.  Demonstrates another use for sewage treatment as producing energy.
VOCAB: (w/definition)	Effluent-liquid waste or sewage discharged into a river or the sea. centrifuges-a machine with a rapidly rotating container that applies centrifugal force to its contents, typically to separate fluids of different densities (e.g. cream from milk) or liquids from solids
Cited references to follow up on	https://www.nyc.gov/site/dep/water/sewer-system.page
Follow up Questions	What happens after the waste is dumped at the landfill? Does this process release harmful gasses and is the water itself completely safe? What are the pros and cons of using methane as a gas source?

Notes:

- Wastewater Treatment Process: The process involves 14 Wastewater Resource Recovery Facilities and undergoes five major treatment processes.
- Preliminary Treatment: Incoming wastewater is screened and pumped, removing debris like plastic and leaves.
- Primary Treatment: Wastewater enters settling tanks where lighter solids are skimmed, and heavier sludge settles for removal.
- Secondary Treatment: Aeration tanks foster microorganisms that consume organic material, followed by final settling to remove heavier solids.
- Disinfection: Sodium hypochlorite disinfects the wastewater before releasing it into local waterways.
- Sludge Treatment: Collected sludge is thickened, digested, and processed into biosolids that can be composted or used in agriculture.
- Waste-to-Energy: Biogas produced during sludge digestion is harnessed for heat and electricity, reducing greenhouse gas emissions.

Article #4 Notes: THE CHALLENGE OF WASTEWATER TREATMENT AROUND THE WORLD

Source Title	THE CHALLENGE OF WASTEWATER TREATMENT AROUND THE WORLD
Source citation (APA Format)	Gagné, J.-S. (2023, August 23). *The challenge of wastewater treatment around the world*. Bio-Sol. https://www.bio-sol.ca/en/the-challenge-of-wastewater-treatment-around-the-world/
Original URL	https://www.bio-sol.ca/en/the-challenge-of-wastewater-treatment-around-the-world/
Source type	Organization article
Keywords	Wastewater, Challenges, Energy in-efficient
#Tags	#brainstorming, #efficiency
Summary of key points + notes (include methodology)	Most developing countries use separate sewage systems which has different pipelines for runoff and sewage-as opposed to conventional systems where it is the same pipeline- but they weren't built to accommodate rapid population growth. As a result, the sewage pipes are being merged with the runoff pipes- which aren't designed to treat sewage water- which causes unfiltered sewage water to mix with the water cycle. This causes a variety of problems such as diseases since this water is being consumed, inability to quantify chemical levels, increases energy cost.
Research Question/Problem/ Need	What are some problems in developing countries related to water/sewage management?
Important Figures	
VOCAB: (w/definition)	
Cited references to follow up on	

Follow up Questions	Why can’t the pipes be extended? Are there any portable filtration systems to purify this water so its safe enough to drink? The article mentions inefficiencies that cause cost to go up, what are those and how can they be fixed?

Notes:

- Less than 8% of wastewater is treated in underdeveloped and developing countries, posing a global challenge.
- Wastewater, if viewed as a solution, requires proper collection, treatment, and disposal methods.
- Pollutants in wastewater include heavy metals, pathogens, plant nutrients, organic pollutants, and micro-pollutants.
- Eutrophication, caused by excessive nutrients, reduces water quality globally, impacting biodiversity.
- Sewerage systems, especially in developing countries, face challenges like cross-connections and lack of maintenance.
- On-site systems have issues with poor construction, illegal dumping, and lack of treatment facilities.
- Untreated wastewater poses health risks, with pathogens causing diseases and contaminating water sources.
- Agricultural wastewater contributes to non-point source pollution, with issues like sediment, nutrient, microbial, and chemical runoff.
- Sludge disposal challenges include land, ocean, and air disposal, with recycling as a viable long-term solution.
- Energy challenges in wastewater management emphasize the need for high-efficiency technologies to reduce costs and emissions.
- Pollutants in wastewater: Heavy metals, pathogens, plant nutrients, organic pollutants, and micro-pollutants.
- Eutrophication impact: Reduced water quality globally by at least one third, worst-hit regions include Europe, China, South Asia, Japan, and Southern Africa.
- Sewerage systems: Conventional/combined and separate systems; separate systems face challenges like cross-connections.
- Collection system challenges: Improper planning, old infrastructure, wrong sizing, lack of resilience, inadequate maintenance, and regulatory gaps.
- On-site system challenges: Poor construction, illegal dumping, lack of municipal services, and absence of sludge treatment facilities.
- Diseases caused by pathogens in wastewater: Bacteria, helminths, protozoa, and viruses causing various diseases
- Agricultural wastewater challenges: Sediment runoff, nutrient runoff, microbial runoff, and chemical runoff contributing to non-point source pollution.

- Sludge disposal strategies: Land, ocean, air disposal; recycling as a long-term solution.
- Energy challenges: High energy consumption in wastewater management, emphasis on biogas and high-efficiency technologies.

Article #5 Notes: Lab-grown meat: the science of turning cells into steaks and nuggets

Source Title	Lab-grown meat: the science of turning cells into steaks and nuggets
Source citation (APA Format)	Jones, N. (2023). Lab-grown meat: the science of turning cells into steaks and nuggets. *Nature*, *619*(7968), 22–24. https://doi.org/10.1038/d41586-023-02095-6
Original URL	https://www-nature-com.ezpv7-web-p-u01.wpi.edu/articles/d41586-023-02095-6
Source type	Journal Article
Keywords	Serum-free medium, Chick myogenic cells, Antibiotics Adipose-derived mesenchymal stem cells, Chemically defined cell culture media, Fetal bovine serum, In vitro methods
#Tags	#brainstorming #ideas
Summary of key points + notes (include methodology)	There are many problems with cultured meat such as high energy use, finding the perfect feed for cells,and finding productive starter cells which prevent the industrialization of lab grown meat. One of the main reasons for such problems is gaps in knowledge as lab grown meat is a fairly new concept. Current predictions show that it will take years to get cultured meat to have the same nutrition, efficiency and even environmental impact(cultured meat is worse in an environmental point of view as of now).
Research Question/Problem/ Need	How does the emerging field of lab-grown meat, cultivated through cellular agriculture, address environmental, health, and sustainability concerns associated with traditional meat production?
Important Figures	
VOCAB: (w/definition)	Coax: arrange (something) carefully into a particular shape or position. Cell-lines : cultures of animal cells that can be propagated repeatedly and sometimes indefinitely.
Cited references to follow up on	Vergeer, R., Sinke, P. & Odegard, I. *TEA of Cultivated Meat: Future Projections of Different Scenarios* (CE Delft, 2021).

	Oxford Martin School. *Meat: The Future Series — Alternative Proteins* (World Economic Forum, 2019).
Follow up Questions	What methods are used to synthesize a feed for the cell cultures? How do current bioreactors work? What is stopping us from making advancements in this field?

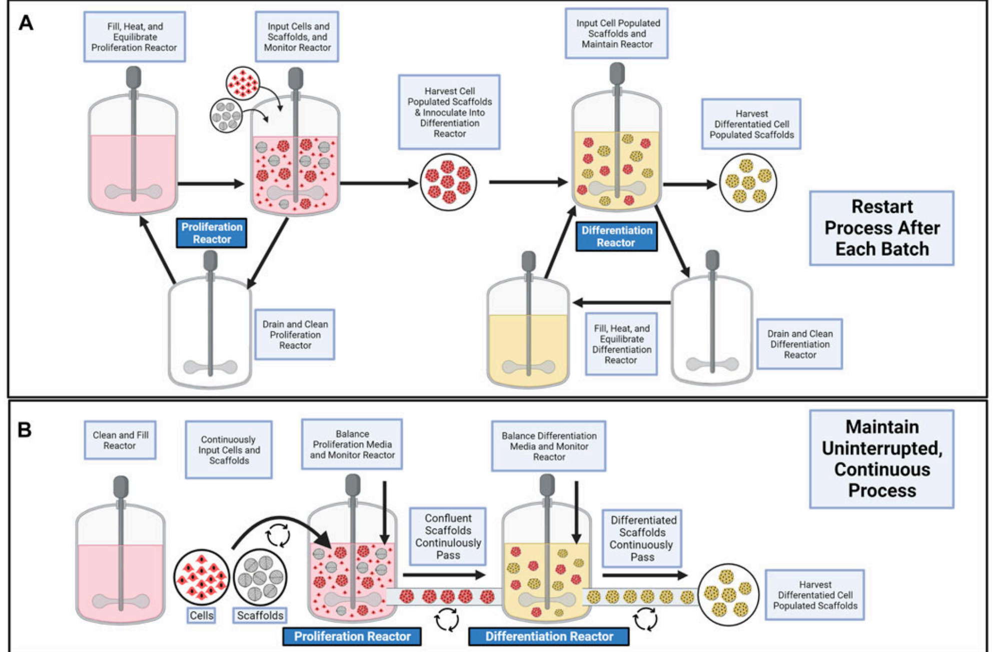
Notes:

- Problems with current regular meat growing
  - Uses lots of land
  - 15% of world's greenhouse gas emissions
  - Known to promote viruses (influenza, avian, and more)
  - Fish farms pollute ocean waters
- Lab grown meat is much more efficient use of land
- Current versions are hundreds times more expensive than regular meat
- Basic Process
  - Take biopsy
  - Nurture cells in nutrients so they multiply
  - Convert cells into muscle or fat cells(give specialization to cells)
  - Exercise muscle cells to build fibers
- Challenges:
  - Finding good started cells
  - Have the most nourishing feed
  - Cost
  - Improve fiber structure
  - Energy use
- Potential ways to improve
  - Experimenting with different started cells and sometimes combining multiple
  - Genetically modify cells
  - Fibroblast cells
    - Easy to grow (found in chickens)
- Fish cells are easier as they are less delicate than mammalian cells
- Feed is the most expensive: amino acids, proteins, sugars, salts, and vitamins

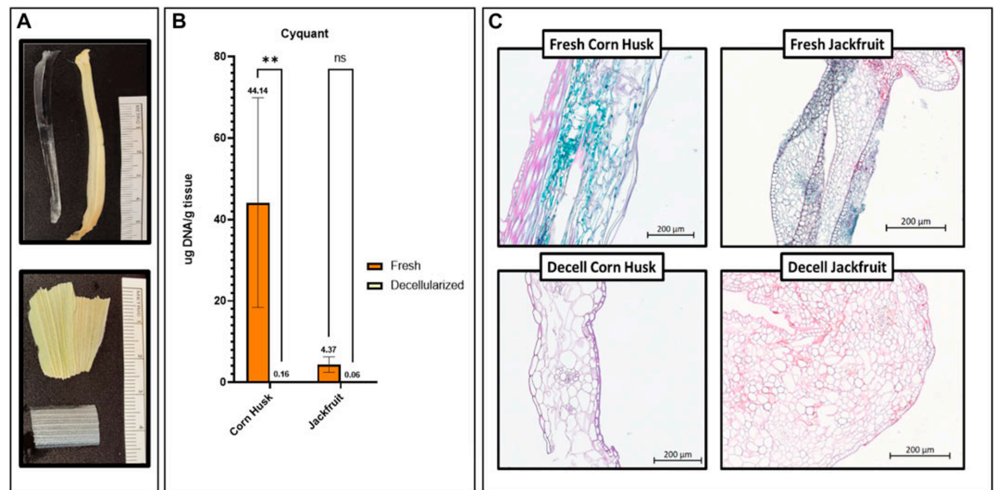
Article #6 Notes: Repurposing agricultural waste as low-cost cultured meat scaffolds

Source Title	Repurposing agricultural waste as low-cost cultured meat scaffolds
Source citation (APA Format)	Perreault, L. R., Thyden, R., Kloster, J., Jones, J. D., Nunes, J., Patmanidis, A. A., Reddig, D., Dominko, T., & Gaudette, G. R. (2023). Repurposing agricultural waste as low-cost cultured meat scaffolds. *Frontiers in Food Science and Technology*, *3*. https://www.frontiersin.org/articles/10.3389/frfst.2023.1208298
Original URL	https://www.frontiersin.org/articles/10.3389/frfst.2023.1208298/full
Source type	Journal Article
Keywords	Cultured meat, biomaterials, cellular agriculture, plant-derived scaffolds, sustainability, taste characteristics, bacterial cellulose, food-grade microcarriers, agricultural waste valorization.
#Tags	#Ideas #introduction
Summary of key points + notes (include methodology)	The study suggests using agricultural waste, specifically corn husks and jackfruit rinds, as viable scaffolds for large-scale cellular agriculture production. Jackfruit, in particular, demonstrates mechanical similarity to native muscle and favorable protein content, making it a promising candidate for a cell-scaffold hybrid meat product. The study found successful results on using waste products as scaffolds.
Research Question/Problem/ Need	How does the utilization of low valued bio products such as corn husk and jackfruit rinds as scaffolds affect cell proliferation?

Important Figures



Shows process of cultured meat and role of scaffolds. Good diagram to explain someone in a basic way.



Shows zoomed in picture of decellularized and regular scaffold replacements. Can see the difference in fiber structure. There are more holes so more opportunities for cells to stick in decellularized scaffolds.

VOCAB: (w/definition)

In vitro: made to occur outside the living organism in an artificial environment, such as a culture medium
Bovine: cattle related
Scaffolds: organic materials that provide a biomimetic environment for stem cells.

Cited references to follow up on

Bar-Shai, N., Sharabani-Yosef, O., Zollmann, M., Lesman, A., and Golberg, A. (2021). Seaweed cellulose scaffolds derived from green macroalgae for tissue engineering. *Sci. Rep.* 11 (1), 11843. doi:10.1038/s41598-021-90903-2
Galiwango, E., Abdel Rahman, N. S., Al-Marzouqi, A. H., Abu-Omar, M. M., and Khaleel,

	A. A. (2019). Isolation and characterization of cellulose and α-cellulose from date palm biomass waste. *Heliyon* 5 (12), e02937. doi:10.1016/j.heliyon.2019.e02937
Follow up Questions	How can the taste characteristics of cell-cultured meat be further optimized for consumer acceptance? What are the key challenges and regulatory considerations in large-scale production of cell-cultured meat? How can the sustainability of agricultural waste valorization for biomaterials production be enhanced?

Notes:

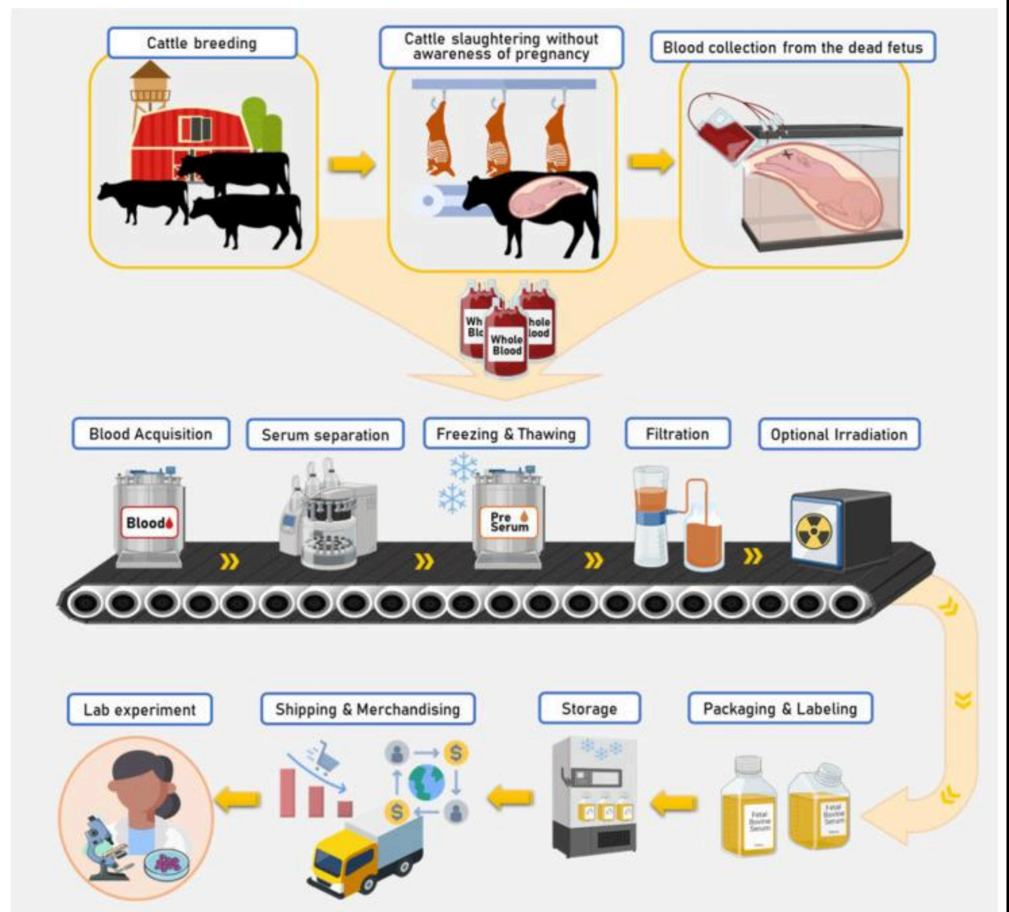
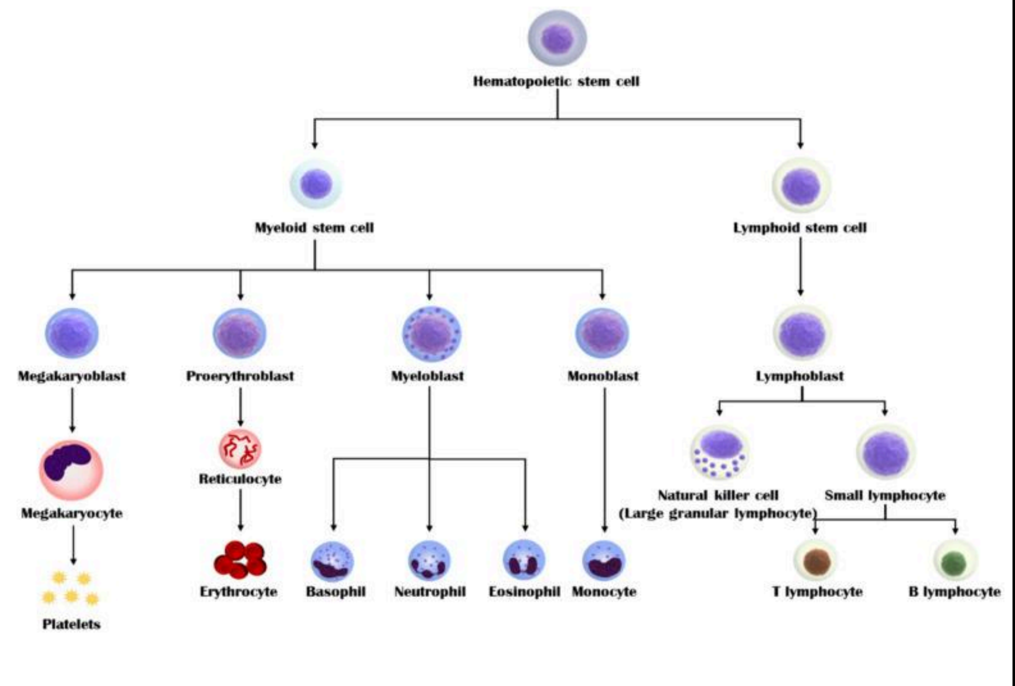
- Corn husk and jackfruit rind are abundant in cellulose and might be able to be used as scaffolds
- The rising world population (projected over 9 billion by 2050) strains current food production systems, exacerbated by limited resources and environmental concerns.
- Cellular Agriculture is proposed as a sustainable alternative, cellular agriculture aims to produce meat in vitro, reducing environmental impact.
- Technological Barriers: Despite potential, technological and economic barriers hinder mass production of cultured meat, requiring low-cost solutions.
- Plant-Based Scaffolds: Edible plant waste, like corn husks and jackfruit rinds, is explored as scaffolds for cultured meat production.
- Plant materials are decellularized to provide safe and nutritious scaffolds for cell growth.
- Meat Alternatives: Jackfruit, with its fibrous texture, proves promising for mimicking structured meat in cellular agriculture.
- Corn Husk Stiffness: Before decellularization: ±6.42 MPa After decellularization: 6.18±2.42 MPa
- Jackfruit Stiffness: Fresh: 7.54±2.42 MPa Decellularized: 2.47±1.47 MPa
- Cell Seeding: BSCs and QM7 cells on decellularized jackfruit and corn husk Adhered extensively, observed for 3 and 7 days
- Protein Content Analysis: Untreated jackfruit scaffold: Negligible PBSC-seeded and QM7-seeded jackfruit samples had higher protein content
- Bead-to-Bead Transfer: Circular corn husk scaffolds had higher nuclei density than square and triangular
- Plant-Based Scaffolds: Corn husk and jackfruit identified as potential sources Stiffness highlighted as a critical factor
- Conclusion: Plant scaffolds from agricultural waste can be affordable and edible for cultured meat Unique characteristics of corn husk and jackfruit make them promising for cellular agriculture Consideration for continuous bioprocess design for cultured meat scale-up
- Biomaterials from waste: Isolating cellulose from date palm biomass and corn cob for potential applications

Notes for video:

365 mil tons of meat per year
120kg meat/person in USA annually
Food production ⅓ of all global emissions of greenhouse gases

Article #7 Notes: Review of the Current Research on Fetal Bovine Serum and the Development of Cultured Meat

Source Title	Review of the Current Research on Fetal Bovine Serum and the Development of Cultured Meat
Source citation (APA Format)	Lee, D. Y., Lee, S. Y., Yun, S. H., Jeong, J. W., Kim, J. H., Kim, H. W., Choi, J. S., Kim, G.-D., Joo, S. T., Choi, I., & Hur, S. J. (2022). Review of the Current Research on Fetal Bovine Serum and the Development of Cultured Meat. *Food Science of Animal Resources*, *42*(5), 775–799. https://doi.org/10.5851/kosfa.2022.e46
Original URL	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9478980/
Source type	Journal Article
Keywords	cultured meat, fetal bovine serum, blood, muscle cells, scaffolds,
#Tags	#Introduction
Summary of key points + notes (include methodology)	The article focuses on studies investigating blood and fetal bovine serum (FBS) in vertebrates, especially in major livestock, and explores current research on commercializing cultured meat. Detailed research on FBS is lacking, and while it consists of proteins, carbohydrates, growth factors, cytokines, fats, vitamins, minerals, hormones, non-protein nitrogen, and inorganic compounds, there is limited understanding of how its composition differs from adult animals' blood or serum. Challenges in developing cultured meat include high production costs and the need to reduce or replace ingredients like FBS due to ethical concerns and safety issues.
Research Question/Problem/ Need	How do different culture conditions and mediums impact the in vitro development and production of embryos, cells, and tissues for various animal species?

Important Figures	 Demonstrates basic process of the process of manufacturing FBS. It is easy to build a base and understand the big picture. 

VOCAB: (w/definition)	
Cited references to follow up on	Bhuiyan MMU, Kang SK, Lee BC. Supplementation of fructose in chemically defined protein-free medium enhances the *in vitro* development of bovine transgenic cloned embryos. *Zygote.* 2007;15:189–198. doi: 10.1017/S0967199407004236. [PubMed] [CrossRef] [Google Scholar] Chelladurai KS, Christyraj JDS, Rajagopalan K, Yesudhason BV, Venkatachalam S, Mohan M, Vasantha NC, Christyraj JRSS. Alternative to FBS in animal cell culture: An overview and future perspective. *Heliyon.* 2021;7:e07686. doi: 10.1016/j.heliyon.2021.e07686. Gstraunthaler G, Lindl T, van der Valk J. A plea to reduce or replace fetal bovine serum in cell culture media. *Cytotechnology.* 2013;65:791–793. doi: 10.1007/s10616-013-9633-8. [PMC free article] [PubMed] [CrossRef] [Google Scholar] Jones JD, Rebello AS, Gaudette GR. Decellularized spinach: An edible scaffold for laboratory-grown meat. *Food Biosci.* 2021;41:100986. doi: 10.1016/j.fbio.2021.100986. [CrossRef] [Google Scholar]
Follow up Questions	1. How do silver nanoparticles affect the immune, redox, and lipid status of chicken blood, and what are the implications for poultry farming? 2. In the context of cultured meat production, how does the use of Chlorella vulgaris extract influence the proliferation and differentiation of bovine myoblasts? 3. What are the key findings regarding the birth of piglets from in vitro-produced porcine blastocysts, and how does the chemically defined medium contribute to this process?

Notes:

- FBS is a main component in artificial meat
- Very expensive and also not practical for industrialization
- Blood composition can vary from animal to animal depending on environmental factors
- FBS used for all in vitro culture of cells

- FBS is used as an adhesion activator for cells to attach to dishes
- Albumin specifically affects adhesion
- Immune response is minimized due to low concentration of immunoglobulin
- Cytokines- induce cell growth
- “FBS contains essential components required for cell attachment, proliferation, and maintenance, such as serum albumin, fetuin, hormones, vitamins, trace elements, and growth factors”
- Γ-globulins- inhibit activity
- Countries are slowly banning FBS
- Not sourced sustainably
- Not immune to viruses
- “high endotoxin content and can be a potential source of microbial contaminants, including fungi, bacteria, viruses, and prions”
- “Serum-free media have been developed to avoid the use of animals as cell culture media and have achieved great success, especially in the production of some proteins for medical use (Cruz et al., 1998; Hawkes, 2015). However, pharmaceutical companies, diagnostic laboratories, and researchers still depend heavily on FBS for most cell culture needs” WHY?

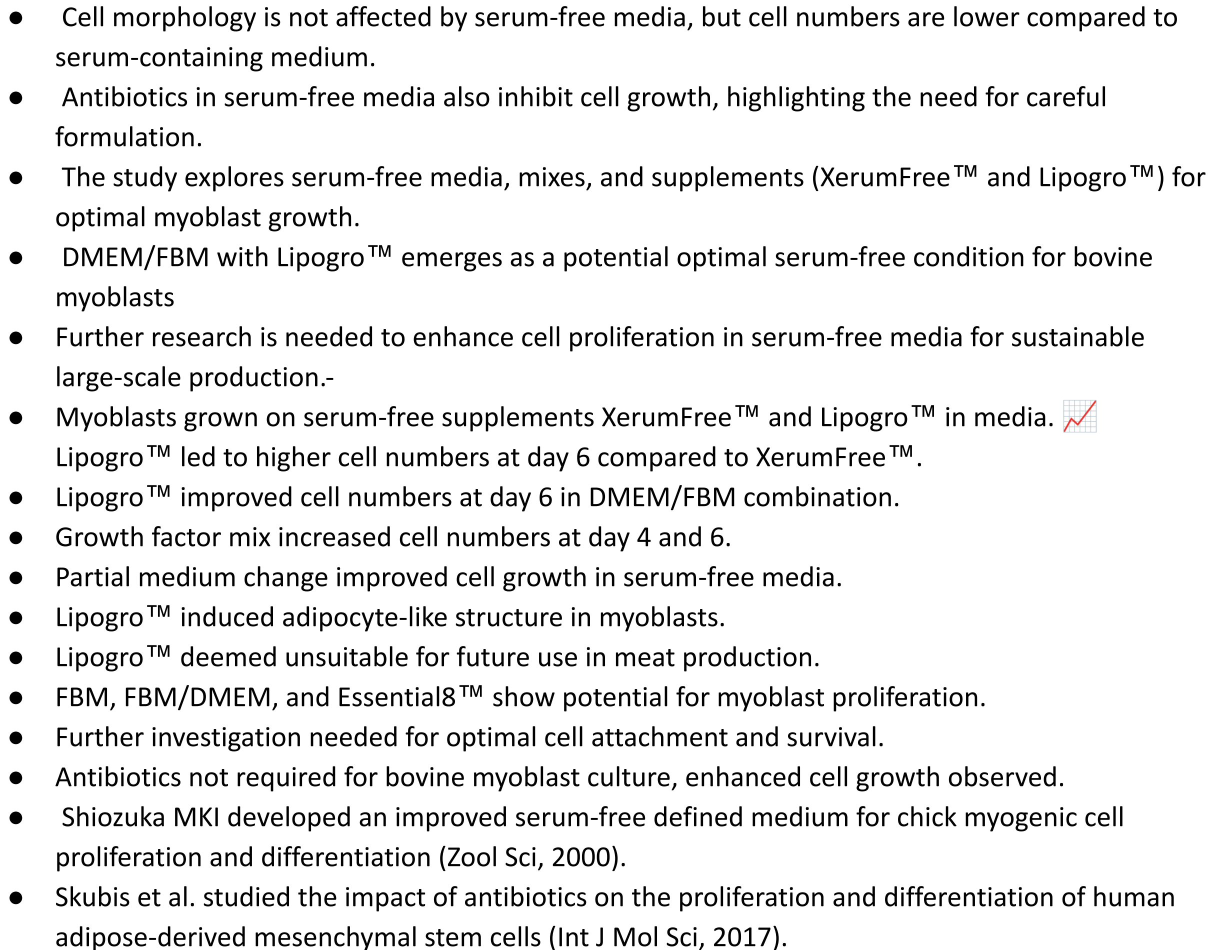
Article #8 Notes: Serum-free media for the growth of primary bovine myoblasts

Source Title	Serum-free media for the growth of primary bovine myoblasts
Source citation (APA Format)	Kolkmann, A. M., Post, M. J., Rutjens, M. A. M., van Essen, A. L. M., & Moutsatsou, P. (2020). Serum-free media for the growth of primary bovine myoblasts. *Cytotechnology*, *72*(1), 111–120. https://doi.org/10.1007/s10616-019-00361-y
Original URL	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7002633/
Source type	Journal Article
Keywords	Myoblasts, Proliferation, Xeno-free media
#Tags	#Introduction #ideas #methods
Summary of key points + notes (include methodology)	The provided articles delve into the influence of distinct cell culture media on cellular behaviors. It focuses on enhancing chick myogenic cells through a serum-free medium. The study explores the impact of antibiotics on human adipose-derived stem cells. The central question revolves around understanding how these media alterations affect cell proliferation and differentiation. Further research could explore the specific components enhancing cell functions, the mechanisms behind antibiotic effects, and the broader applications of optimized in vitro methods.
Research Question/Problem/ Need	How can cell culture media be optimized for effective proliferation and differentiation of specific cell types?
Important Figures	Shows that the current alternatives are lacking very behind FBS. there is a lot more

	research to be done to get a productive alternative.
VOCAB: (w/definition)	Myoblasts- stem cells containing skeletal muscle cell precursors DMEM- Dulbecco's Modified Eagle Medium is a widely used basal medium for supporting the growth of many different mammalian cells xeno-free media- growth medium without animal and human derived components
Cited references to follow up on	Allen RE, Dodson MV, Luiten LS, Boxhorn LK. A serum-free medium that supports the growth of cultured skeletal muscle satellite cells. *In Vitro Cell Dev Biol.* 1985;21:636–640. doi: 10.1007/BF02623296. [PubMed] [CrossRef] [Google Scholar] Gstraunthaler G. Alternatives to the use of fetal bovine serum: serum-free cell culture. *Altex.* 2003;20:275–281. [PubMed] [Google Scholar] Laitinen A, et al. A robust and reproducible animal serum-free culture method for clinical-grade bone marrow-derived mesenchymal stromal cells. *Cytotechnology.* 2016;68:891–906. doi: 10.1007/s10616-014-9841-x. [PMC free article] [PubMed] [CrossRef] [Google Scholar]
Follow up Questions	What are the key components of the improved serum-free defined medium for chick myogenic cells developed by Shiozuka MKI? How do antibiotics affect the proliferation and differentiation of human adipose-derived mesenchymal stem cells, as studied by Skubis et al.? In the context of van der Valk et al.'s research, what alternatives to fetal bovine serum were explored, and what were the observed outcomes in mammalian in vitro methods?

Notes:

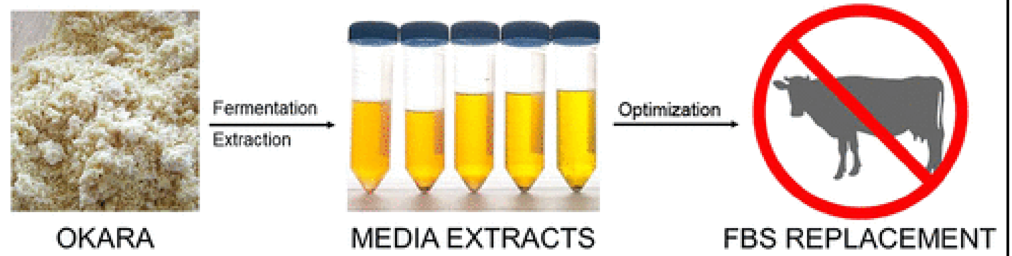
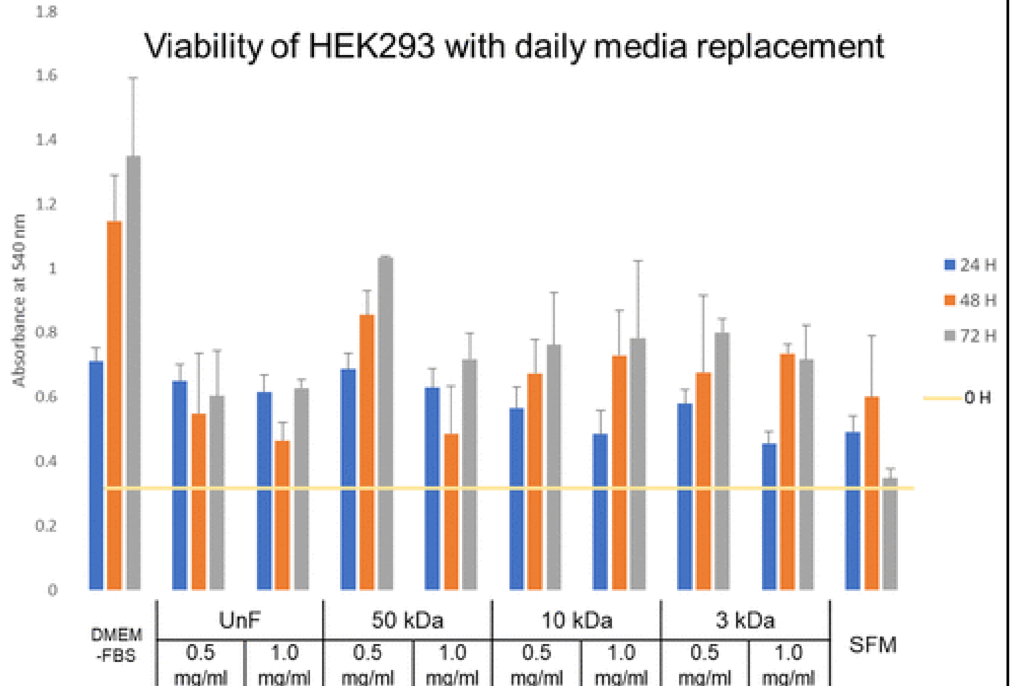
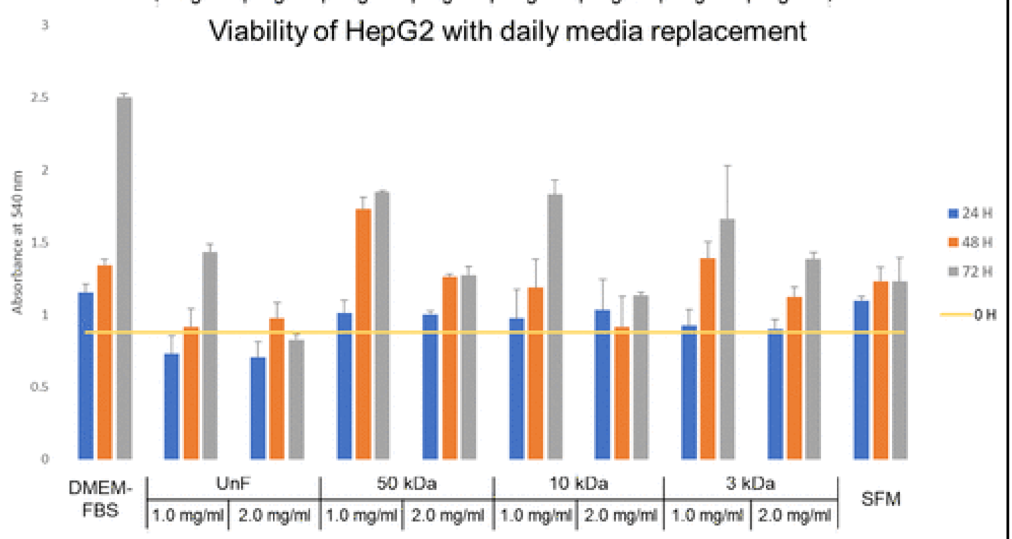
- The demand for meat is outpacing livestock production capacity.
- Cultured beef is a potential alternative, but sustainability concerns arise from using fetal bovine serum.
- Researchers aim to find a serum-free medium for culturing bovine myoblasts.
- Commercial xeno-free media like FBM™, TesR™, and Essential 8™ show potential for bovine myoblast proliferation.
- XenoFree™ and a custom growth factor mix fail to stimulate cell proliferation effectively.
- Serum-free media stimulate cell expansion, but not as much as the current method with up to 30% serum.
- Transition to serum-free media is crucial for large-scale biotechnological production, especially for cultured meat.
- The study explores primary bovine myoblast culture with a focus on serum replacements.
- Antibiotics in serum-containing medium reduce cell proliferation, prompting investigation into serum-free media
- FBM, Essential8™, and TESR-E8™ show consistent cell growth in serum-free conditions over 6 days.



- Cell morphology is not affected by serum-free media, but cell numbers are lower compared to serum-containing medium.
- Antibiotics in serum-free media also inhibit cell growth, highlighting the need for careful formulation.
- The study explores serum-free media, mixes, and supplements (XerumFree™ and Lipogro™) for optimal myoblast growth.
- DMEM/FBM with Lipogro™ emerges as a potential optimal serum-free condition for bovine myoblasts
- Further research is needed to enhance cell proliferation in serum-free media for sustainable large-scale production.-
- Myoblasts grown on serum-free supplements XerumFree™ and Lipogro™ in media. 📈 Lipogro™ led to higher cell numbers at day 6 compared to XerumFree™.
- Lipogro™ improved cell numbers at day 6 in DMEM/FBM combination.
- Growth factor mix increased cell numbers at day 4 and 6.
- Partial medium change improved cell growth in serum-free media.
- Lipogro™ induced adipocyte-like structure in myoblasts.
- Lipogro™ deemed unsuitable for future use in meat production.
- FBM, FBM/DMEM, and Essential8™ show potential for myoblast proliferation.
- Further investigation needed for optimal cell attachment and survival.
- Antibiotics not required for bovine myoblast culture, enhanced cell growth observed.
- Shiozuka MKI developed an improved serum-free defined medium for chick myogenic cell proliferation and differentiation (Zool Sci, 2000).
- Skubis et al. studied the impact of antibiotics on the proliferation and differentiation of human adipose-derived mesenchymal stem cells (Int J Mol Sci, 2017).

Article #9 Notes: Ultrafiltrated Extracts of Fermented Okara as a Possible Serum Alternative for Cell Culturing: Potential in Cultivated Meat Production

Source Title	Ultrafiltrated Extracts of Fermented Okara as a Possible Serum Alternative for Cell Culturing: Potential in Cultivated Meat Production
Source citation (APA Format)	Teng, T. S., Lee, J. J. L., & Chen, W. N. (2023). Ultrafiltrated Extracts of Fermented Okara as a Possible Serum Alternative for Cell Culturing: Potential in Cultivated Meat Production. *ACS Food Science & Technology*, *3*(4), 699–709. https://doi.org/10.1021/acsfoodscitech.2c00401
Original URL	https://doi.org/10.1021/acsfoodscitech.2c00401
Source type	Journal Article
Keywords	animal serum, cultivated meat production, okara extracts
#Tags	#methods #analysis #ideas
Summary of key points + notes (include methodology)	Animal serum such as fetal bovine serum (FBS) is widely used in cell culture media, but it has several drawbacks. Fermented okara extract is a potential serum alternative that is more ethical, biosafe, and cost-effective. The authors of the study found that the 50 kDa fractionated fermented okara extract at 1 mg/mL was the most effective serum replacement for HEK293 and HepG2 cells. The extract also supported the growth of C2C12 and immortalized porcine myoblast cultures. These results suggest that ultrafiltrated potential fermented okara extract is a promising novel serum alternative for cellular agriculture use.
Research Question/Problem/ Need	Can ultrafiltrated fermented okara extract be a viable alternative to traditional serum in cellular agriculture?

Important Figures	   Shows performance of a serum free medium okara extract
VOCAB: (w/definition)	enzymatic hydrolysis solid-state fermentation-biotransform agro-wastes for nutritional enhancement precision fermentation-microbes being programmed to produce compounds that are otherwise found only in animals

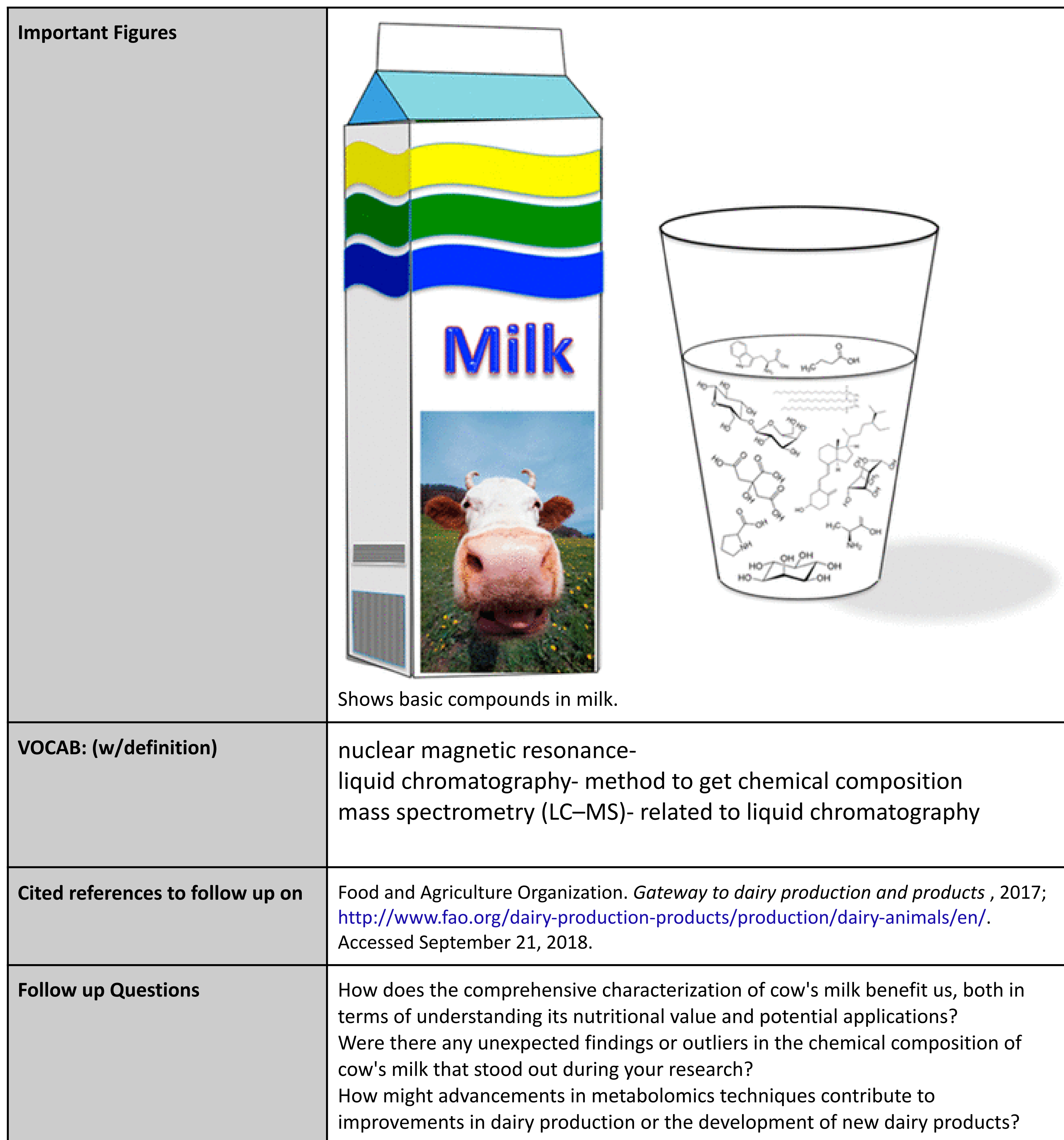
	HEK293-human kidney cell line HepG2-Human liver cancer cell line C2C12-cell line derived from mouse muscle cells. It is used to study muscle development and function, IPM cells-Interphotoreceptor Matrix cell. It is a type of cell that is found in the retina of the eye. MTT assay- test to determine cell viability
Cited references to follow up on	Analysis of Improved Nutritional Composition of Potential Functional Food (Okara) after Probiotic Solid-State Fermentation
Follow up Questions	What specific metabolites in the fermented okara extract contribute to cell viability? How does the cost of using fermented okara extract compare to traditional FBS in large-scale production? Are there any potential regulatory hurdles or safety concerns associated with the use of fermented okara extract in cell culture?

Notes:

- The use of animal serum in cell culturing media poses ethical, biosafety, and supply issues.
- Serum, especially fetal bovine serum (FBS), is costly and may introduce contaminants.
- Plant Protein Hydrolysates Studies explore plant protein hydrolysates as an alternative to animal serum.
- Hydrolysis breaks down plant proteins into peptides, offering nutrients and bioactivities for cell growth.
- Okara as a Resource Okara, soybean residue, is rich in protein but often wasted due to microbial putrefaction. Solid-state fermentation transforms okara into valuable plant protein extracts with advantages in scalability, reproducibility, and cost.
- Okara Extracts in Cultivated Meat Production Extracts from fermented okara are proposed as a serum alternative for cultivated meat production. Different fractions (50 kDa, 10 kDa, 3 kDa) show varying effectiveness in promoting cell viability.
- Materials and Methods Okara fermentation and extraction methods followed established protocols. Cell lines (HEK293, HepG2, C2C12, IPM) were cultured with different fractions of fermented okara extracts.
- Okara extracts, especially in 50 kDa and 10 kDa fractions, show promise as serum alternatives for cell cultures.
- In a nutshell, the study explores using fermented okara extracts as a sustainable and effective alternative to animal serum in cell culture, especially for applications like cultivated meat production.

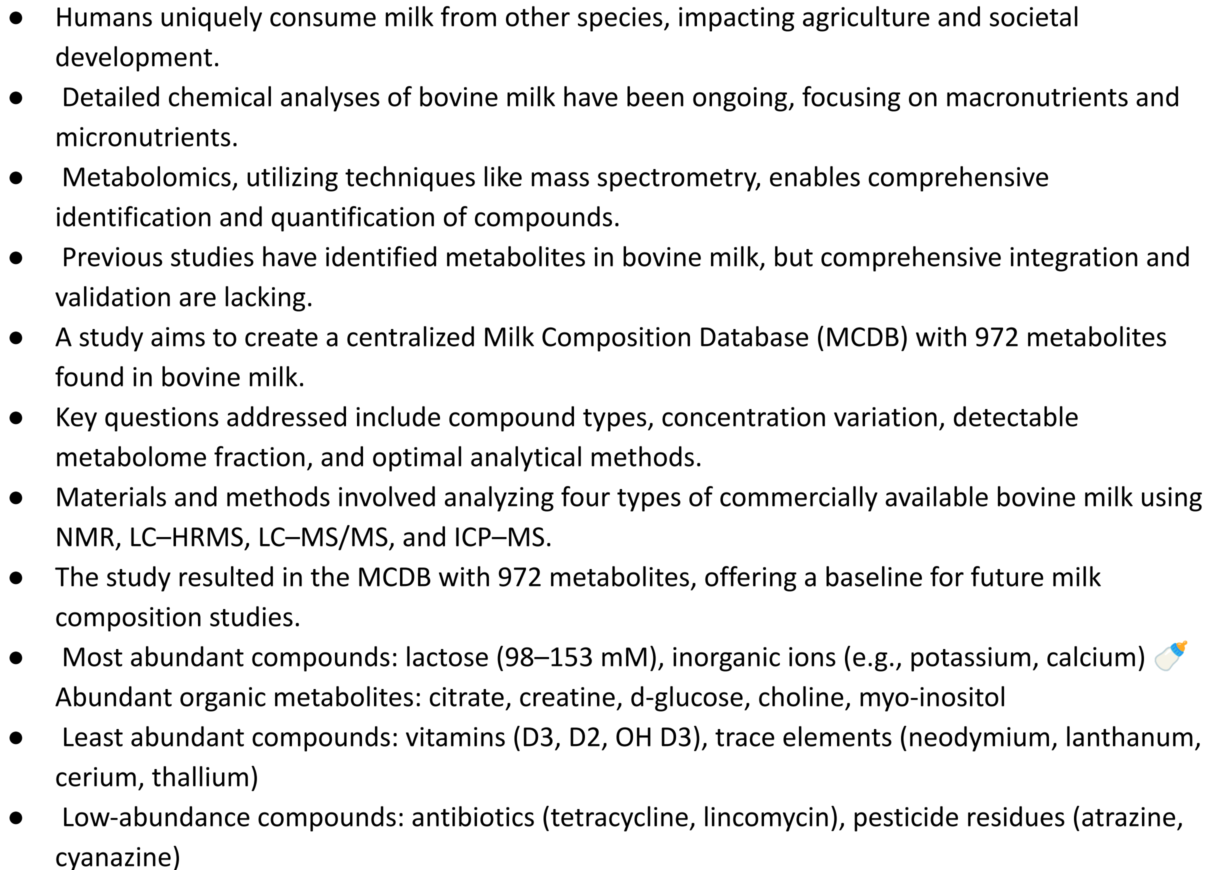
Article #10 Notes: Chemical Composition of Commercial Cow's Milk

Source Title	Chemical Composition of Commercial Cow's Milk
Source citation (APA Format)	Foroutan, A., Guo, A. C., Vazquez-Fresno, R., Lipfert, M., Zhang, L., Zheng, J., Badran, H., Budinski, Z., Mandal, R., Ametaj, B. N., & Wishart, D. S. (2019). Chemical Composition of Commercial Cow's Milk. *Journal of Agricultural and Food Chemistry*, *67*(17), 4897–4914. https://doi.org/10.1021/acs.jafc.9b00204
Original URL	https://doi.org/10.1021/acs.jafc.9b00204
Source type	Journal Article
Keywords	milk metabolomics NMR LC−MS ICP−MS literature review chemical composition
#Tags	#Introduction #methods #ideas
Summary of key points + notes (include methodology)	The authors of this study used modern metabolomics techniques to obtain the most comprehensive and up-to-date characterization of the chemical constituents in commercial cow's milk. They identified and quantified 296 bovine milk metabolites or metabolite species (corresponding to 1447 unique structures) from a variety of commercial milk samples. Through their literature analysis, they also found another 676 metabolites or metabolite species (corresponding to 908 unique structures). This study provides the most comprehensive and up-to-date information on the chemical composition of commercial cow's milk.
Research Question/Problem/ Need	What are all the chemical compounds present in COmmercial cow's milk?

Important Figures	 Shows basic compounds in milk.
VOCAB: (w/definition)	nuclear magnetic resonance- liquid chromatography- method to get chemical composition mass spectrometry (LC–MS)- related to liquid chromatography
Cited references to follow up on	Food and Agriculture Organization. *Gateway to dairy production and products* , 2017; http://www.fao.org/dairy-production-products/production/dairy-animals/en/. Accessed September 21, 2018.
Follow up Questions	How does the comprehensive characterization of cow's milk benefit us, both in terms of understanding its nutritional value and potential applications? Were there any unexpected findings or outliers in the chemical composition of cow's milk that stood out during your research? How might advancements in metabolomics techniques contribute to improvements in dairy production or the development of new dairy products?

Notes:

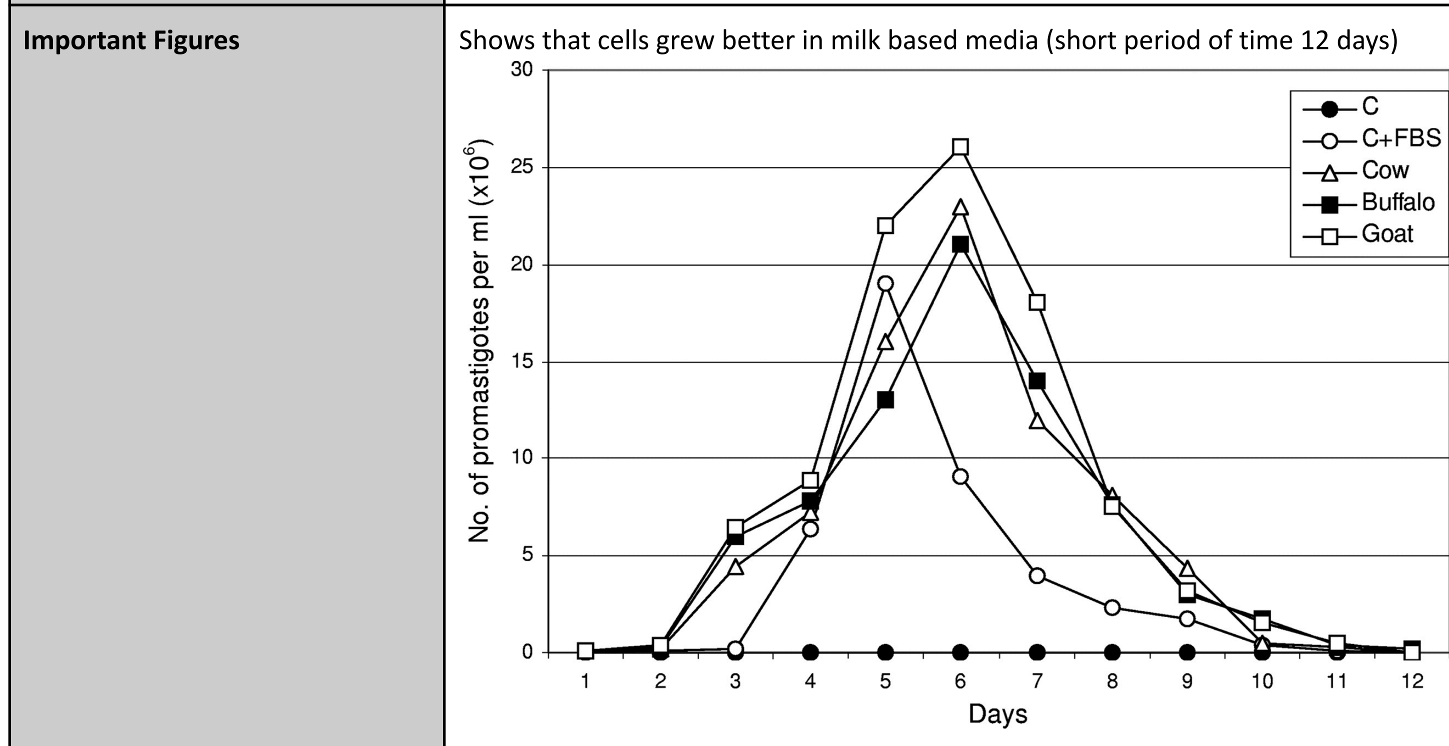
- Milk is considered the "perfect food" with key nutrients adjusted for newborn mammals.
- Global milk production is dominated by cows (83%), followed by buffaloes, goats, sheep, and camels.

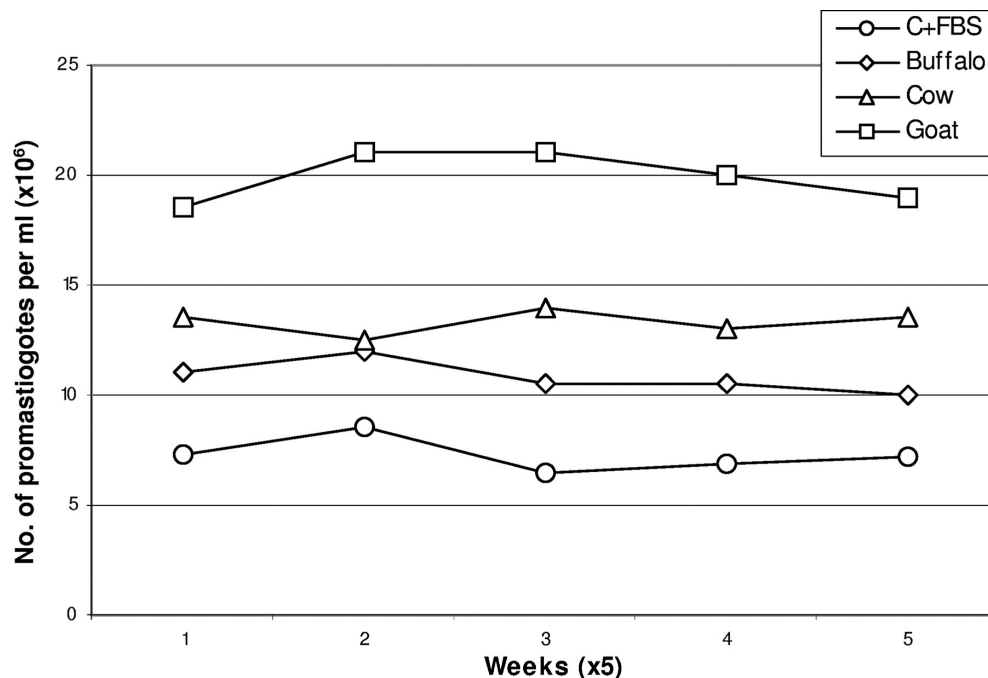


- Humans uniquely consume milk from other species, impacting agriculture and societal development.
- Detailed chemical analyses of bovine milk have been ongoing, focusing on macronutrients and micronutrients.
- Metabolomics, utilizing techniques like mass spectrometry, enables comprehensive identification and quantification of compounds.
- Previous studies have identified metabolites in bovine milk, but comprehensive integration and validation are lacking.
- A study aims to create a centralized Milk Composition Database (MCDB) with 972 metabolites found in bovine milk.
- Key questions addressed include compound types, concentration variation, detectable metabolome fraction, and optimal analytical methods.
- Materials and methods involved analyzing four types of commercially available bovine milk using NMR, LC–HRMS, LC–MS/MS, and ICP–MS.
- The study resulted in the MCDB with 972 metabolites, offering a baseline for future milk composition studies.
- Most abundant compounds: lactose (98–153 mM), inorganic ions (e.g., potassium, calcium) 🍼 Abundant organic metabolites: citrate, creatine, d-glucose, choline, myo-inositol
- Least abundant compounds: vitamins (D3, D2, OH D3), trace elements (neodymium, lanthanum, cerium, thallium)
- Low-abundance compounds: antibiotics (tetracycline, lincomycin), pesticide residues (atrazine, cyanazine)

Article #11 Notes: Milk of Cow (Bos taurus), Buffalo (Bubalus bubalis), and Goat (Capra hircus): a Better Alternative than Fetal Bovine Serum in Media for Primary Isolation, In Vitro Cultivation, and Maintenance of Leishmania donovani Promastigotes

Source Title	Milk of Cow (Bos taurus), Buffalo (Bubalus bubalis), and Goat (Capra hircus): a Better Alternative than Fetal Bovine Serum in Media for Primary Isolation, In Vitro Cultivation, and Maintenance of Leishmania donovani Promastigotes
Source citation (APA Format)	Muniaraj, M., Lal, C. S., Kumar, S., Sinha, P. K., & Das, P. (2007). Milk of cow (bos taurus), Buffalo (bubalus bubalis), and goat (Capra Hircus): A better alternative than fetal bovine serum in media for primary isolation, in vitro cultivation, and maintenance of leishmania donovani promastigotes. *Journal of Clinical Microbiology*, *45*(4), 1353–1356. https://doi.org/10.1128/jcm.01761-06
Original URL	https://journals.asm.org/doi/full/10.1128/jcm.01761-06
Source type	Journal Article
Keywords	Tyndalized milk, Leishmania donovani, Fetal bovine serum (FBS), Parasite culture, Primary isolation, Biochemical investigation, Alternative to FBS
#Tags	#Milk #Ideas
Summary of key points + notes (include methodology)	Researchers explored the use of tyndalized milk from goats, cows, and buffaloes as a substitute for fetal bovine serum (FBS) in cultivating Leishmania donovani promastigotes. Results showed that milk-supplemented media supported parasite growth, with goat milk being the most effective. Milk supplementation

	demonstrated a remarkable increase in cell density compared to FBS, making it a potential, cost-effective alternative for parasite culture. The tyndalization process, a simple method to sterilize milk, proved effective in preventing contamination for up to 6 months. This study suggests that milk supplements could simplify the diagnosis of leishmaniasis, reduce the need for FBS, and alleviate ethical concerns related to animal suffering. Further research is needed to explore the broader utility of this approach and standardize milk samples.
Research Question/Problem/ Need	Can tyndalized milk from goats, cows, and buffaloes serve as a viable and cost-effective alternative to fetal bovine serum (FBS) in the cultivation, primary isolation, and maintenance of Leishmania donovani promastigotes?
Important Figures	Shows that cells grew better in milk based media (short period of time 12 days) 

	 Shows that cells grew better in milk based media (long period of time 5 weeks)
VOCAB: (w/definition)	Leishmania donovani promastigotes- a parasite Tyndallization- version of pasteurization phosphate-buffered salt solution- used to wash cells Hemocytometer- used to count cells Nalgene- a type of filter NNN medium- rabbit blood based medium
Cited references to follow up on	Chaudhuri, G., K. Ghoshal, S. Sen, S. Pal., and A. B. Banerjee. 1986. Nutrition of Leishmania donovani donovani: growth in new semidefined & completely chemically defined media. Indian J. Med. Res.84:461-468. Viander, B., S. Ala-Uotila, M. Jalkanen, and R. Pakkanen. 1996. Viable AC-2, a new adult bovine serum- and colostrum-based supplement for the culture of mammalian cells. BioTechniques20:702-707.
Follow up Questions	What specific growth factors or components in tyndalized milk contribute to superior performance in supporting the growth of Leishmania donovani promastigotes compared to traditional fetal bovine serum (FBS)? How does the efficacy of different types of milk, such as goat, cow, and buffalo, vary in transmitting amastigotes during primary isolation, and what factors influence their effectiveness in parasite culture? Considering the ethical and cost-related concerns associated with the use of FBS,

	how can the findings of this study be practically applied in the development of more accessible and humane in vitro cultivation methods for other organisms?

Notes:

Background and Introduction:

- Tyndalized milk from goats, cows, and buffaloes explored as a substitute for fetal bovine serum (FBS) in cultivating Leishmania donovani promastigotes.
- FBS is traditionally used in media for parasitic protozoa cultivation, but issues include cost, contamination risk, and ethical concerns.

Objectives of the Study:

- Investigate the use of common cattle milk as a replacement for FBS in primary isolation, cultivation, and maintenance of L. donovani parasites.

Parasite and Milk Samples:

- L. donovani parasites isolated from splenic/bone marrow aspirates of kala-azar/visceral leishmaniasis patients.
- Milk samples collected from cow, buffalo, and goat, subjected to tyndalization for sterilization.

Tyndalization Process:

- Milk subjected to fractional sterilization method called tyndalization.
- Process involves exposing milk to boiling water on successive days to eliminate contaminants, followed by centrifugation to remove fat globules.

Culture Methods:

- Promastigotes sedimented, washed, and adjusted to 1 × 10^6 parasites/ml.
- Inoculation of tubes with medium 199 alone, medium 199 + 10% FBS, and medium 199 + 10% milk (cow, buffalo, goat) for up to 12 days at 25°C.
- Monitoring growth through daily microscopy.

Primary Isolation:

- Aspirates from 26 patients inoculated into NNN medium and medium 199 with tyndalized milk.
- Milk-supplemented media showed 84.6% positivity for promastigotes, outperforming NNN medium.

Maintenance of Culture:

- Weekly subpassaging of promastigote culture in medium 199 + 10% milk (cow, buffalo, goat) for up to 25 subpassages.

Biochemical Investigation:

- Tyndalized and centrifuged milk samples and FBS examined for protein, glucose, triglycerides, calcium, and cholesterol.
- Goat milk showed highest protein (5.8 g/dl) and triglycerides (485.8 mg/dl), buffalo milk highest in calcium (42.4 mg/dl) and cholesterol (70.7 mg/dl).
- Glucose level exceptionally higher in FBS (156 mg/dl).

Results and Conclusions:

- Milk-supplemented media showed superior growth of promastigotes compared to FBS.
- Goat milk demonstrated the highest efficacy in transmitting amastigotes.

- Tyndalized milk stored at 3 to 4°C remained uncontaminated for 6 months.
- Milk supplement suggested as a cost-effective, easy-to-implement alternative to FBS in Leishmania culture.
- Biochemical analysis indicated key factors influencing growth, with potential applications in simplifying diagnostic and culture work for leishmaniasis.

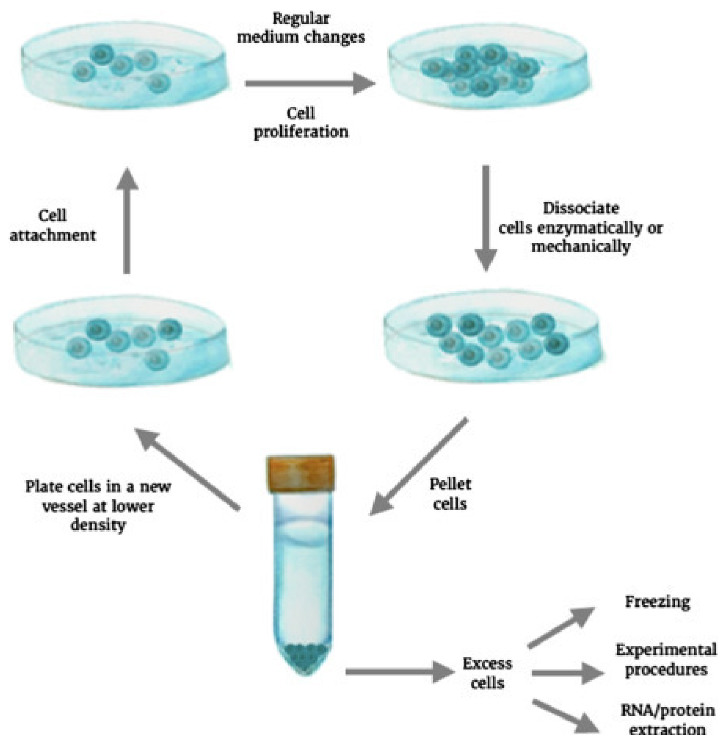
Future Recommendations:

- Further research needed to explore the utility of milk supplements for other Kinetoplastida.
- Standardization required for locally available milk samples.
- Minor drawbacks noted, such as haziness of medium, warranting further investigation.

Article #12 Notes: Cell Culture

Source Title	Cell Culture
Source citation (APA Format)	Segeritz, C.-P., & Vallier, L. (2017). Cell culture. *Basic Science Methods for Clinical Researchers*, 151–172. https://doi.org/10.1016/b978-0-12-803077-6.00009-6
Original URL	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7149418/#:~:text=In%20a%20clinical%20context%2C%20however,toxicity%20of%20novel%20drug%20compounds.
Source type	Journal Article
Keywords	Cell culture, asepsis, primary cell, contamination, medium, supplements, incubator, biosafety level, hazard group, maintenance
#Tags	#Introduction #Methodology
Summary of key points + notes (include methodology)	Cell culture stands as a versatile and invaluable tool in scientific research, offering insights into fundamental biological processes, disease mechanisms, and drug toxicity. However, ensuring the safety of both lab personnel and the integrity of the research itself is paramount. Rigorous safety measures are indispensable to prevent exposure to hazardous agents, and adherence to established guidelines is crucial for both laboratory personnel and facility design. Aseptic practices, including the use of biosafety cabinets and appropriate equipment, play a vital role in maintaining a sterile cell culture environment. Swift detection and elimination of contaminations, ranging from bacteria and yeast to molds and viruses, are imperative to mitigate risks and preserve the reliability of experimental outcomes. In the dynamic realm of cell culture, a proactive approach to safety and contamination control is essential for robust and meaningful scientific investigations.
Research Question/Problem/ Need	How can cell culture techniques be applied to address basic and translational research questions?

Important Figures



Visual representation of the culture process

Table 9.2

Recommended Equipment for the Cell Culture Laboratory

Equipment	Purpose
Biosafety cabinet	– To create sterile work surface; class II and III recommended
Humid CO_2 incubator	– To provide a physiological environment for cellular growth
Inverted light microscope	– To assess cell morphology and count cells
Fridge, freezers (−20°C, −80°C), liquid nitrogen storage	– To store cells, cell material, and culture components
Centrifuge	– To condense cells
pH meter	– To determine the correct pH of media components
Pipettes and pipettors	– To aliquot different volumes
Cell media and supplementary components	– To culture cells in desirable components
Hemacytometer	– To count cells, determine growth kinetics and prepare suitable plating densities
Autoclave	– To sterilize pipettes and other equipment in contact with cells
Vacuum pump	– To aspirate cell culture medium
Water bath (with adjustable temperature)	– To warm up cell culture media
Cell culture dishes	– To culture cells in different formats (e.g., flasks, Petri dishes, 96-well plates)
Containers for waste (biohazardous)	– To correctly dispose of waste

Shows important items needed for safety

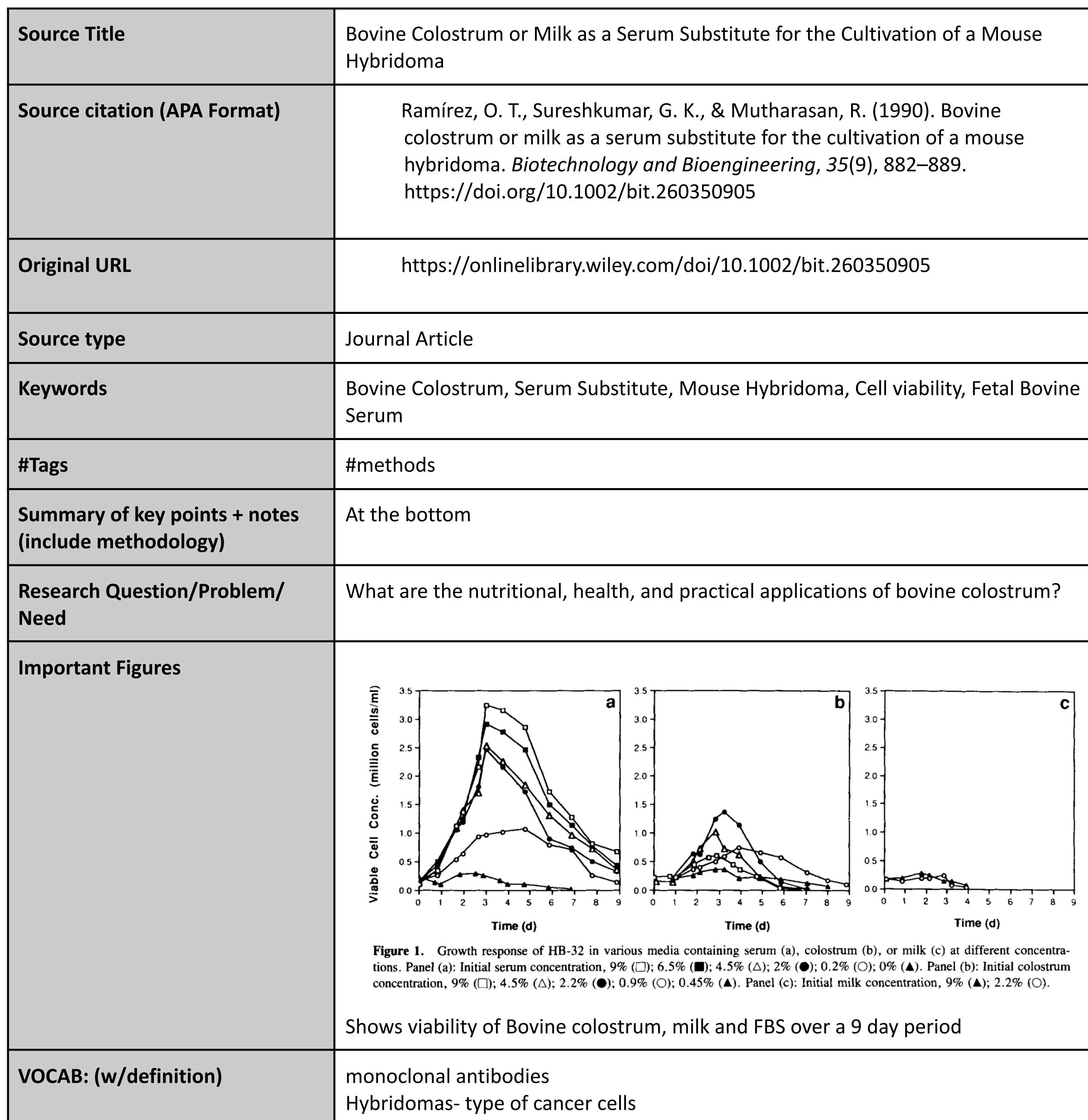
VOCAB: (w/definition)

clonal cell populations

	asepsis—the creation of a microenvironment free of unwanted pathogenic microorganisms, including bacteria, viruses, fungi, and parasites skin biopsies Hepatocyte fibroblasts
Cited references to follow up on	Strober W. Trypan blue exclusion test of cell viability. *Current Protocols in Immunology, Appendix 3B.* 2001
Follow up Questions	How do researchers overcome the challenges of oversimplification in in vitro cellular environments? What are some examples of specific genes studied through genetic engineering in cell cultures?

Notes:

Article #13 Notes: Bovine Colostrum or Milk as a Serum Substitute for the Cultivation of a Mouse Hybridoma

Source Title	Bovine Colostrum or Milk as a Serum Substitute for the Cultivation of a Mouse Hybridoma
Source citation (APA Format)	Ramírez, O. T., Sureshkumar, G. K., & Mutharasan, R. (1990). Bovine colostrum or milk as a serum substitute for the cultivation of a mouse hybridoma. *Biotechnology and Bioengineering*, *35*(9), 882–889. https://doi.org/10.1002/bit.260350905
Original URL	https://onlinelibrary.wiley.com/doi/10.1002/bit.260350905
Source type	Journal Article
Keywords	Bovine Colostrum, Serum Substitute, Mouse Hybridoma, Cell viability, Fetal Bovine Serum
#Tags	#methods
Summary of key points + notes (include methodology)	At the bottom
Research Question/Problem/ Need	What are the nutritional, health, and practical applications of bovine colostrum?
Important Figures	 **Figure 1.** Growth response of HB-32 in various media containing serum (a), colostrum (b), or milk (c) at different concentrations. Panel (a): Initial serum concentration, 9% (□); 6.5% (■); 4.5% (△); 2% (●); 0.2% (○); 0% (▲). Panel (b): Initial colostrum concentration, 9% (□); 4.5% (△); 2.2% (●); 0.9% (○); 0.45% (▲). Panel (c): Initial milk concentration, 9% (▲); 2.2% (○). Shows viability of Bovine colostrum, milk and FBS over a 9 day period
VOCAB: (w/definition)	monoclonal antibodies Hybridomas- type of cancer cells

	Ampules trypan blue exclusion- Type of Assay
Cited references to follow up on	Sources were too old
Follow up Questions	Can other cholostrums be used as a serum replacement? What would happen if FBS was combined with Colostrum? How would colostrum affect non-mammalian cell cultures?

Notes:

- Mouse-mouse hybridoma grown in serum-free medium with bovine milk or colostrum.
- Bovine colostrum supports hybridoma growth; bovine milk alone does not support proliferation.
- Maximum cell concentration in 2.2% colostrum is 1.4 x 10^6 cells/mL, 44% of 9% serum.
- In 2.2% milk with 0.4% serum, max cell concentration is 2 x 10^6 cells/mL, better than milk or serum alone.
- Similar trends observed in colostrum and low serum conditions.
- Monoclonal antibodies (MAb) crucial in biology and medicine.
- Traditional hybridoma growth in serum-supplemented medium (10-20%).
-
- Nutrients, hormones, binding proteins, attachment factors, enzymes.
- Serum reduction enhances monoclonal antibody production economics.
- Two approaches: defined element supplementation and unconventional serum substitutes.
- Drawbacks of defined elements: cell line specificity, expensive process.
- Human plasma byproducts, enzymatic digests, amniotic and spinal fluid.
- Challenges: limited availability, minimal improvement over serum.
- Similarities with serum: binding proteins (lactalbumin, transferrin), mitogenic factors, enzymes, inhibitors.
- Rich in nutrients (carbohydrates, lipoproteins, vitamins, trace elements).
- Presence of macrophages, T lymphocytes, and B lymphocytes.
- Cost-effective and readily available.
- Unexplored potential for hybridoma growth.
- Successful cultivation with fibroblasts and epithelial cells.
- Additional requirements for surface-dependent cell growth.
- Supports mouse-mouse hybridoma growth without compromising MAb production.
- Reduces serum requirements in cell culture significantly.
- Mouse-mouse hybridoma: 14-4-43 (ATCC No. HB-32).
- Produces cytotoxic monoclonal antibodies (IgG2a, kappa).
- Reacts with I-Ek/Ck determinants.
- Objective: Investigate the potential of bovine colostrum as a serum substitute.
- Focus on growth support for the mouse-mouse hybridoma (14-4-43).
- Monoclonal antibody production without compromising viability.

Processing Steps:

- Freezing: Both milk and colostrum frozen within four hours after milking.
- Thawing: Thawed as needed.
- Centrifugation: High-speed centrifuge (SS3-Automatic, Ivan Sorvall Inc., Newtown, CT) at 13,000 rpm for 30 min at room temperature.
- Top layer (fat) and bottom layer (cells and sediments) discarded.

Filtration Process:

- Dilution: Milk and colostrum diluted with Dulbecco's Modified Eagle's Medium (DMEM) at various concentrations.
- Prefiltration: Coarse filter used as an initial step.
- Sterile Filtration: Through 0.45- and 0.22-µm filters.
- Only colostrum dilutions less than 9% (v/v) passed through the filters.

Storage:

- Sterile DMEM supplemented with milk or colostrum stored for a maximum of 24 hours at 4°C before use.

Growth Experiment Conditions:

- Conducted in 100 mL T-flasks.
- CO_2 incubator maintained at 37°C with 6% CO_2.
- To minimize passage number variations, experiments initiated from stock ampules at the same passage number stored in liquid nitrogen.

Cell Preparation:

- Ampule Thawing: Thawed ampules before inoculation.
- Passage Conditioning: Transferred twice in DMEM supplemented with fetal bovine serum (9% by volume).
- Centrifugation: Cells at mid to late exponential phase centrifuged at 3000 rpm for 10 min.
- Resuspension: Discarded supernatant, and cells resuspended in fresh DMEM without fetal bovine serum.

Inoculation and Culture Conditions:

- Inoculum adjusted to 0.2 x 10^6 cells/mL to ensure consistent starting conditions.
- Media supplemented with varying concentrations of serum (0-9%), milk (0-9%), colostrum (0-9%), conditioned medium, or combinations of these.
- Daily cell counts in duplicate using a hemocytometer, and viability determined by trypan blue exclusion.
- Daily samples frozen for later monoclonal antibody (MAb) concentration analysis.

Monoclonal Antibody Determination:

- Analysis of MAb concentration done on the last two samples of each growth experiment.
- Alkaline phosphatase "sandwich" ELISA technique used.
- Procedure:
    - Sheep anti-mouse IgG adsorbed to wells.
    - Blocking with 1% bovine serum albumin in PBS.

  - Addition of standards and culture supernatant (diluted 1:800).
  - Reaction with alkaline phosphatase labeled sheep anti-mouse IgG.
  - Addition of p-nitrophenyl phosphate.
  - Reaction stopped with NaOH after 20 min.
  - Reading at 405 nm on an ELISA plate spectrophotometer.
- MAb titers reported from the average of 12 wells.
-

Article #14 Notes: Colostrum and its benefits: a review

<table>
<tr><td>Source Title</td><td>Colostrum and its benefits: a review</td></tr>
<tr><td>Source citation (APA Format)</td><td>Uruakpa, F. O., Ismond, M. A. H., & Akobundu, E. N. T. (2002). Colostrum and its benefits: A Review. Nutrition Research, 22(6), 755–767. https://doi.org/10.1016/s0271-5317(02)00373-1</td></tr>
<tr><td>Original URL</td><td>https://www.sciencedirect.com/science/article/pii/S0271531702003731?casa_token=QZs3tfSn12QAAAAA:eqKFX9Skgjy6AIsoGrXIdk6YsmqoOnoa4y2VC3akTcCTXFepe9RtUZeWlSOz8xRe4WoT0zjHuA</td></tr>
<tr><td>Source type</td><td>Journal Article</td></tr>
<tr><td>Keywords</td><td># Introduction #methods</td></tr>
<tr><td>#Tags</td><td>Human milk, Colostrum, Immunity Wound healing, Growth factors</td></tr>
<tr><td>Summary of key points + notes (include methodology)</td><td>Colostrum, a nutrient-rich fluid produced by mammals after birth, is a potent source of immune, growth, and tissue repair factors. Bovine colostrum, in particular, has applications in treating gastrointestinal infections. It contains essential components such as immunoglobulins, lactoferrin, and growth factors that contribute to immune support, wound healing, and muscular-skeletal repair.</td></tr>
<tr><td>Research Question/Problem/ Need</td><td>What are the nutritional, health, and practical applications of bovine colostrum?</td></tr>
<tr><td>Important Figures</td><td>Table 1. Cellular contents of colostrum

<table>
<tr><th>Cell type</th><th>Mean number ± SEM (per mm³)</th><th>Percentage of total cells</th></tr>
<tr><td>Macrophages</td><td>2860 ± 1166</td><td>49</td></tr>
<tr><td>Polymorphs</td><td>1964 ± 855</td><td>37</td></tr>
<tr><td>Lymphocytes</td><td>675 ± 312</td><td>12</td></tr>
<tr><td>Epithelial cells</td><td>98 ± 41</td><td>2</td></tr>
</table>
Reprinted from Chandra RK, Breast Feeding: Immunologic and Nutritional Considerations, Clinical Nutrition, 2: 21–24, 1983, by permission of the publisher Churchhill Livingstone.</td></tr>
<tr><td>VOCAB: (w/definition)</td><td>Lactoferrin</td></tr>
</table>

	Secretory IgA ultrafiltrate-supplemented medium Lactoferrin - in colostrum has antibacterial and antiviral properties.
Cited references to follow up on	C.L. Berseth, L.M. Lichtenberger, F.H. Morris Comparison of the gastrointestinal growth-producing effects of rat colostrum and mature milk in newborn rats in vivo Am J Clin Nutr, 37 (1983), pp. 52-58
Follow up Questions	How does bovine colostrum compare to other milk sources, particularly in terms of protein, fat, and carbohydrate content? In what ways is bovine colostrum utilized in cell cultures, and what are its specific effects on different types of cells? How often has bovine colostrum been tested on cell cultures and can it be a viable alternative for FBS and is it as versatile as FBS?

Notes:

- Colostrum is a rich source of immune, growth, and tissue repair factors.
- It plays a vital role in developing immunity in newborns.
- Colostrum is a potent natural immune booster, containing immunoglobulins and complement components.
- Human milk, especially colostrum, provides significant immune protection to infants.
- Secretory IgA in breast milk acts as the first line of defense against infections.
- Colostrum contains growth factors crucial for muscle and cartilage repair.
- Transforming growth factors and insulin-like growth factors are found exclusively in colostrum.
- Bovine colostrum is richer in immune factors, particularly IgG, than human colostrum.
- It has applications in preventing and treating infections of the digestive tract.
- Topically applied colostrum shows effectiveness against Chlamydia trachomatis and eye dryness.
- Colostrum contains proline-rich polypeptide (PRP) that stimulates T-cell activation.
- PRP acts as an immunoregulator with anti-inflammatory properties.
- Colostrum is a nutritional supplement, especially beneficial for the elderly.
- Hyperimmune colostral preparations may play a significant role in healthcare.
- Colostrum shows promise in reducing gastrointestinal damage induced by non-steroidal anti-inflammatory drugs (NSAIDs).
- These findings highlight the diverse benefits of colostrum, from immune support to potential applications in healthcare and as a food supplement.
- Influences cell growth, migration, and reduces gut injury.
- Bioenervi supports growth during strenuous physical activity.
- Colostrum's role in wound healing and muscle/cartilage repair.
- Transforming growth factors (TGF-A, TGF-B), insulin-like growth factors (IGF-1, IGF-2) play key roles.
- TGF-beta-2-related growth factor inhibits maternal immune reaction.
- Colostral IGF-1 promotes lean muscle tissue, bone growth, and fat burning.
- IGF-1 infusions lead to significant body weight gain in rats.

- Hepatocyte growth factor (HGF) in colostrum induces intestinal cell growth.
- Whey proteins from buffalo colostrum promote bifidobacteria growth.
- Colostrum can be used as a serum substitute in cell cultures.
- Colostrum rich in nutrients, biologically active molecules.
- Potential for immune system reconstitution, cell growth, tissue repair.
- Biotechnological interest in reproducing colostrum factors.
- Bovine colostrum: Potential health benefits and applications
- Bovine colostrum is rich in growth factors and antimicrobial factors.
- Hyperimmune cow colostrum reduces diarrhea due to rotavirus.
- Increases serum IGF-1 concentration in male athletes during short-term training.

Article #15 Notes: Biologically Active Factors in Bovine Milk and Dairy Byproducts: Influence on Cell Culture

Source Title	Biologically Active Factors in Bovine Milk andDairy Byproducts: Influence on Cell Culture
Source citation (APA Format)	Guimont, C., Marchall, E., Girardet, J. M., Linden, G., & Otani, H. (1997). Biologically active factors in bovine milk and dairy byproducts: Influence on cell culture. *Critical Reviews in Food Science and Nutrition*, *37*(4), 393–410. https://doi.org/10.1080/10408399709527780
Original URL	https://www.tandfonline.com/doi/epdf/10.1080/10408399709527780?needAccess=true
Source type	Journal Article
Keywords	bovine milk, serum, whey proteins, mitogenic factors, cell culture, serum replacement, casein
#Tags	#Introduction #methods #conclusion
Summary of key points + notes (include methodology)	Serum, a complex mixture of molecules, contains essential elements released by cells or obtained from the diet, influencing cell behavior. Growth inhibitors like TGF-β coexist with necessary diffusible and substrate signals for both normal and transformed cells. Various methods, including counting and flow cytometry, are employed to assess cell proliferation. Growth factors like EGF, IGF-1, and PDGF in bovine milk play pivotal roles in specific cell types, while lactoferrin exhibits dual effects on cell growth. Additionally, milk proteins, including p-lactoglobulin, influence cellular behavior and have affinity for ions, impacting cell culture conditions and potentially offering protective effects.
Research Question/Problem/ Need	What are compounds in bovine milk and how does it affect different types of cells in cell cultures?

Important Figures

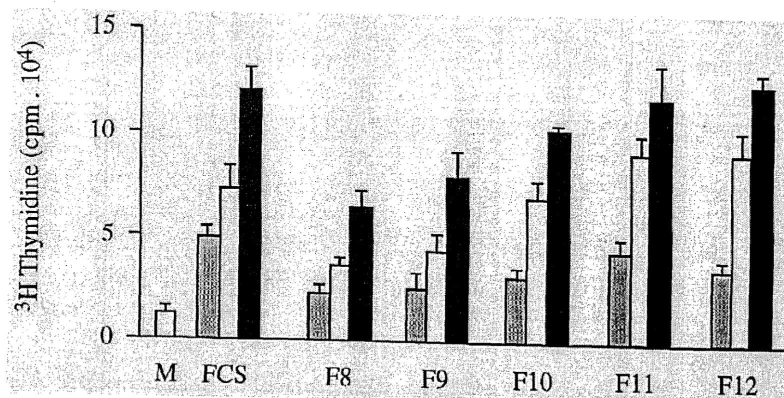


FIGURE 2. DNA synthesis in hybridomas cultured in medium alone (M) and in medium supplemented with fetal calf serum (FCS) or whey fractions (F) in concentrations ranging from 5% (shaded box) to 10% (□) and 20% (■). F7 retentate after ultrafiltration (15°C), concentrated six times (F8); F7 retentate after ultrafiltration (50°C), concentrated six times (F9); F7 retentate after ultrafiltration (50°C), concentrated 12 times (F10); F7 retentate after ultrafiltration (50°C), heated (60 min, 65°C) and concentrated six times (F11); F7 retentate after ultrafiltration (50°C), heated (60 min, 65°C) and concentrated 12 times (F12).

TABLE 1
Hormone in Whey or Mature Bovine Milk

Hormones	Source	Target cells/tissues
Insulin	Mature milk[54,55] Pasteurized milk[54] Sweet whey[43]	Mammary, intestinal, bronchial epithelia, granulosa, ovocytes, mammary cells, fetus, chondrocytes[2,5,18–20,25,50,54,55]
Bombesin	Mature milk[51,53] Boiled milk[53] Whey and commercially instant non-fat dry milk[2]	Gastric, pancreatic epithelium[2,53,54]
Prolactin PRL	Mature milk[2,5,54] Heated milk whey[43,54] Sweet whey[43]	Mammary cells, hybridoma Immunomodulation[2,4,25,43,50,54]
Growth hormone GH	Mature milk[54]	Pituitary gland, milk production[5,25,55]
Adrenocorticotropin ACTH	Sweet and acid whey[43]	Murine, human hybridomas[42,43,55]
Progesterone	Milk[2,55]	Murine hybridoma, mammary cells[25,43,55]
Second messenger cAMP	Sweet and acid whey[43] Bacterial (yogurt) whey[47]	Second cellular cell messenger[25]

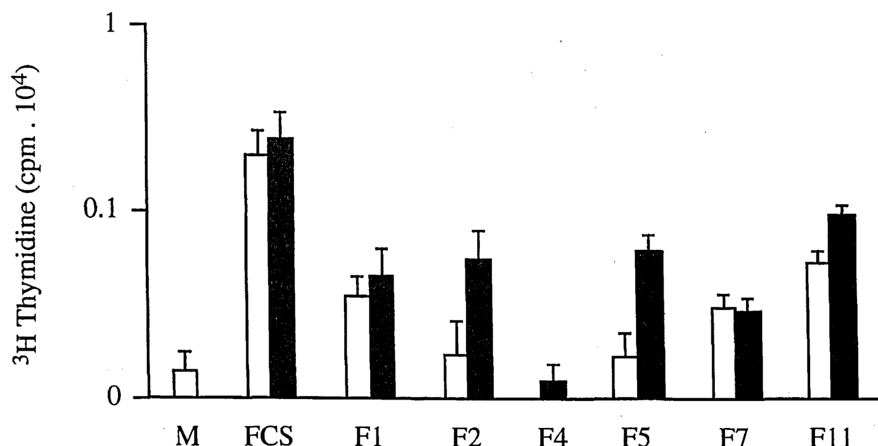
TABLE 3
Bovine Milk Proteins with Cell Growth Activity

Proteins	Source	Target cells/tissues
α, β, κ caseins	Enzymatic or bacterial hydrolysates[46,47,69,70,73]	Fibroblasts, human hybridoma, colon cells, splenocytes, lymphocytes[69–78]
Serum albumin	Acid whey[79]	Lymphocytes, murine, human hybridoma[79,94,97]
β-Lactoglobulin	Sweet and acid whey[83,85]	T lymphocytes, murine hybridoma, mammary epithelial cells[82,83,85]
Proteose peptone 3	Sweet and acid whey[84]	Murine hybridoma[84]
Lactoferrin	Sweet and acid whey[82,90]	Mammary epithelia, lymphocytes, myoblasts, promonocytes, fibroblasts, bacteria, human, murine hybridoma[82,88–95]

Shows components in milk, their purpose and what type of cells need them

TABLE 2
Growth Factors in Whey or Mature Bovine Milk

Hormones	Source	Target cells/tissues
Insulin-like growth factor I IGF-I	Mature milk[51,54,55,61] Heated milk[5]	Mammary, bronchial, intestinal epithelia, ovocytes, granulosa, fetus, chondrocytes, fibroblasts, adipocytes, mesenchyma, dental pulp[2,3,5,15,17–19,22–24,26–28]
Platelet-derived growth factor PDGF	Mature milk[51,63]	Mammary cells, fibroblasts, smooth muscle dental pulp, neural cells[3,23,49,63]
Acidic fibroblast growth factor aFGF	Cheese whey[65]	Fibroblasts, mesodermis, neuroectodermis, epithelial cells, prostate gland[3,51,65]
Basic fibroblast growth factor bFGF	Cheese whey[65]	Fibroblasts, vessels, mammary cells, chondrocytes, myoblasts[3,22,52,65]
Transforming growth factor beta TGF, β1, β2	Pasteurized milk	Fibroblasts, endothelium, B and T lymphocytes[3,21,27,29,30,66]
Nerve growth cell NGF	Mature milk[67]	Neural cells[51,67]
Epidermal growth factor EGF	Mature milk[2,11,51,54,64] Pasteurized milk[54,61]	Intestinal, pulmonary, mammary epithelia, ovocytes, fetal myoblasts, chondrocytes[3,11,14,16,17,21,27,50,51,51]



Shows DNA content of cess after growing for a week in different GM

VOCAB: (w/definition)

granulosa-a type of cell in ovaries that produce hormones including estrogen and progesterone

Mitogenic factors - substances that stimulate cell division and growth.

Epithelial cells- cells that line the surfaces of organs and tissues, including the intestines.

Hybridomas - cells that are created by fusing a specific type of immune cell with a cancer cell, and they are used in research to produce antibodies.

Transformed cells - cells that have undergone genetic changes and have acquired the ability to grow uncontrollably, often seen in cancer cells

	Fibroblasts- type of cell that is involved in the production of connective tissue and wound healing. Fibronecti- protein that plays a role in cell adhesion and is important for the growth and function of fibroblasts. Bombesin- a hormone that can stimulate the division of epithelial cells, which are cells that line the surfaces of organs and tissues. Caseins- main proteins found in milk and are responsible for its white color and nutritional value. IGF-I - insulin-like growth factor I, which is a hormone that promotes cell growth and division. PDGF - platelet-derived growth factor, which is involved in wound healing and tissue repair. EGF - epidermal growth factor, which plays a role in the growth and development of various tissues, including the skin and intestines. aFGF and bFGF- proteins that are known to stimulate the growth of fibroblasts, which are a type of cell involved in tissue repair and wound healing. TGF-pi and TGF-P2- types of transforming growth factors. Mutagenic amines- substances that can cause changes in DNA and potentially affect cell proliferation. Lactoferrin (LF)- protein that binds and transports iron in the body. It is found in various biological fluids, including milk, saliva, and tears. Isomerization- change of structure of a protein Proteolysis- the breakdown of proteins or peptides into amino acids by the action of enzymes.
Cited references to follow up on	Read, L. C., Milk growth factors. In: Cockburn, F.,Ed., Fetal and Neonatal Growth. John Wiley & Sons,London, 1988; 131-152. Murakami, H., Serum-free media used for cultivation of hybridomas. In: Liss, A. R., Ed., Monoclonal An-tibodies: Production and Application. 1989; 107-141. Mossman, J., Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, J. Immunol. Methods, 1983; 65:1-2, 55-63.
Follow up Questions	How would the milk have to be treated before introducing it to cell cultures? Will the cells go through a shock if they are immediately placed in a medium that they aren't used to? How will non mammalian cells react to colostrum based GM?

Notes:

- In resting cells, there is a regulatory mechanism that controls the transition from a quiescent state to cell proliferation
- In vertebrates, the initiation of cell multiplication, known as the "point of no return" or "start" (R point), occurs between the Gl and S phases of the cell cycle
- The conversion from a quiescent state to proliferation is regulated by extracellular signals, such as hormones, neurotransmitters, or growth factors.

- In the case of eukaryotic cells, the culture medium contains essential ingredients that support cell survival, such as carbohydrates, mineral salts, ions, amino acids, and vitamins
- Additionally, serum from young individuals, like calf serum or fetal calf serum (FCS), is added to the culture medium at concentrations ranging from 5% to 15%
- Serum provides soluble elements for all cells and acts as a means of transport for intercellular messages
- Serum is rich in molecules released by cells in the donor or provided by the diet, including trace elements, cellular enzymes, vitamins, metabolites, lipids and their derivatives, hormones, transport proteins, cell adhesion molecules, proliferation factors, and differentiation factors
- Serum also contains growth inhibitors, such as transforming growth factor-p (TGF-p) or growth factor-binding proteins
- For normal cells, two types of signals are necessary: diffusible signals and signals given by a specific substrate or contact with another cell
- Similarly, transformed cells also require these signals, but they may produce these factors themselves and be independent of the external medium for control.
- Several methods are used to determine cell proliferation.For growing cells, counting can be done directly under a microscope or using a counte
- Adherent cells can be quantified using colorimetric or enzymatic techniques
- To measure the response of a cell population to mitogenic stimulation, a labeling index is determined, which represents the percentage of cells in the S-phase during exposure to a labeled precursor like [3H]-thymidine
- Flow cytometry is a technique that allows the observation of the distribution of the cell population based on the amounts of DNA
- EFG (Epidermal Growth Factor) is a mitogenic factor that is found in very small amounts (<2 ng/ml) in bovine milk
- EFG is considered the most important growth factor in human milk
- EFG has specific actions on cells in the stomach, colon, and pancreas.It has been shown to have a direct action on the intestinal epithelium
- During the first days of life, high concentrations of IGF-1 (Insulin-like Growth Factor 1) in the milk coincide with greater permeability of the calf intestine
- Insulin and IGF-1 have a stimulating effect on the growth of bovine bronchial epithelial cells.
- Insulin and IGF-1 also stimulate the proliferation of granulosa cells
- EGF has been shown to promote cell division in bovine fetal myoblasts (muscle precursor cells), while insulin and IGF-1 are positive regulators of fetal growth
- On the other hand, TGF-β (transforming growth factor beta) inhibits the mitogenic (cell division-promoting) response of bovine fetal myoblasts.
- IGF-1, nerve growth factor (NGF), and PDGF (platelet-derived growth factor) have been reported to influence the proliferation of nervous cells and cells derived from dental pulp in bovine origin.
- These growth factors likely play a role in promoting cell division and growth in these specific cell types.
- The growth-promoting effects of bovine colostrum or milk do not extend to fibroblasts because they lack fibronectin.

- The degree of milk protein proteolysis (breakdown of proteins) was found to be directly related to the activation of spleen cells. This means that the more the milk proteins were broken down during fermentation, the greater the activation of spleen cells.
- cAMP can play a role as a positive regulator of cell function and proliferation.
- In bovine milk, IGF-I is attached to a milk protein. However, under acidic conditions (such as in the stomach), this complex is broken apart.
- Human milk and colostrum, which are produced by humans, have higher levels of EGF compared to bovine milk.
- TGF-pi and TGF-P2 have been found to be active in promoting the growth of fibroblasts, endothelial cells (cells that line the interior of blood vessels), B and T lymphocytes (types of immune cells), and ascite cells (cells found in certain types of tumors).
- The study found that lactoferrin (LF) and a mixture of caseins and whey proteins, isolated from sterilized milk, were able to stimulate the proliferation of human and murine hybridomas.
- proteins also have some mitogenic activity, but it is not as strong as lactoferrin and the protein mixture.
- Numerous studies have suggested that caseins or their derived peptides play a major role in modulating the growth of various types of cells, including immune cells.
- Specifically, fragments with low molecular weight (1200 to 2000 Da) and a specific fragment of p-casein (177 to 183) were found to have this stimulatory effect.
- Asl-casein was found to inhibit the production of IgG and IgM by lymphocytes in culture.
- The results suggest that K-casein, due to its protein and glucoside composition, can modulate the proliferation of activated lymphocytes.
- Serum albumin, a protein found in blood plasma, was used as a growth and transport factor for these lymphocytes.
- P-lactoglobulin can bind to and transport retinoic acid, influencing the behavior of lymphocytes.
- The binding of mutagenic amines by P-lactoglobulin may have an impact on the growth and behavior of cells.
- P-lactoglobulin and serum albumin does not influence the growth of epithelial cells.
- LF can act as a growth factor, promoting the proliferation of certain cell types, such as human myoblasts and promonocyte
- inhibitory effect of LF on cell growth, such as the growth of bovine mammary epithelial cells.
- biological properties of LF suggest that it can have both stimulatory and inhibitory effects on cell growth, depending on the specific cell type and context.
- LF has been shown to modulate immune responses, such as the proliferation of blood mononuclear cells and cytotoxic T-lymphocytes.
- Heating milk causes protein denaturation, alterations in mineral balance, and the Maillard reaction.
- Major proteins in milk, such as p-lactoglobulin, cc-lactalbumin, serum albumin, lactoferrin, immunoglobulins, and caseins, can be denatured or hydrolyzed upon heating.
- Proteose-peptones, on the other hand, remain stable under heat.
- Prior binding of ligands to proteins can increase their resistance to thermal denaturation.
- For example, heat-treated bovine holo-lactoferrin was found to support the growth of a cell line.

- Similarly, p-lactoglobulin bound to retinol (vitamin A) showed resistance to moderate thermal denaturation
- Heating milk includes reactions on lactose such as isomerization (changing the structure of lactose) and hydrolysis (breaking down lactose into smaller components).
- At high temperatures, lactose combines with amino acids through a process called the Maillard reaction.
- Some lactose derivatives have mutagenic activities, meaning they can cause changes in DNA
- lactose derivatives can inhibit the growth of fibroblasts, a type of cell involved in tissue repair, through the action of a compound called 3-deoxyglucosone.

β-Lactoglobulin

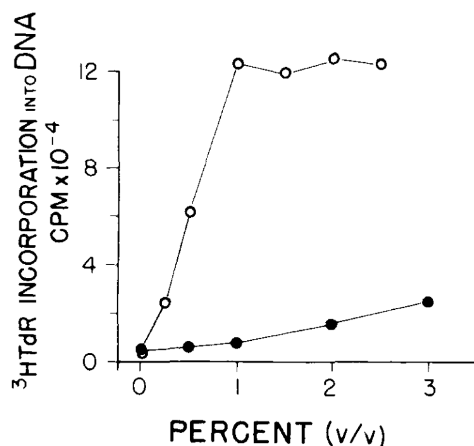
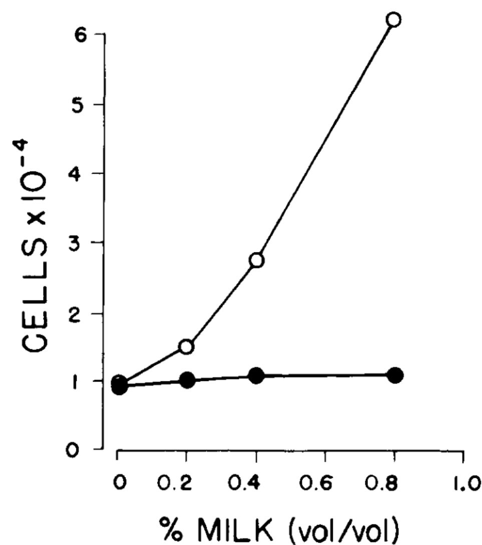
- It carries hydrophobic substances such as lipids, fatty acids, and retinol.
- p-Lactoglobulin exhibits structural homology with retinol-binding protein, which means it has a similar molecular structure.
- There are two variants of p-Lactoglobulin, A and B, which have been shown to exhibit different mitogenic activities in vitro.
- The text suggests that p-Lactoglobulin may have an affinity for Cu^{2+} ions, as it remains strongly bonded to Cu^{2+} under various conditions.
- It is not clear whether p-Lactoglobulin transports Cu^{2+} or Ca^{2+} ions, and further research may be needed to determine this.
- The affinity of p-Lactoglobulin for Mn^{2+}, Zn^{2+}, Mg^{2+}, Ca^{2+}, and Cu^{2+} decreases at lower pH and higher ionic strength, except in the case of Cu^{2+}, which remains strongly bonded regardless of the conditions.
- p-Lactoglobulin has good protein affinity for minerals in cell culture media with a pH of 7.2.
- The viscosity of p-Lactoglobulin may help reduce cell damage, providing a protective effect.
- conformation of P-lactoglobulin can change depending on the polarity of solvents and the presence of phospholipids. Phospholipids are a type of lipid molecule that make up the cell membranes.
- It has specific regions on its structure called antigenic sites that can be recognized by T lymphocytes, which are a type of immune cells.

Proteose-peptone

- The proteose-peptone fraction is a group of proteins that make up 10 to 20% of the proteins found in serum (the liquid part of blood).
- can be dissolved in an acidic solution and are not easily denatured by heat.
- Similarity in PP3 component and murine glycosylation-dependent cell adhesion molecule-1 (GlyCAM-1), which is a protein involved in cell adhesion.

Article #16 Notes: The Serum-Free Growth of Balb/c 3T3 Cells in Medium Supplemented With Bovine Colostrum

Source Title	The Serum-Free Growth of Balb/c 3T3 Cells in Medium Supplemented With Bovine Colostrum
Source citation (APA Format)	Klagsbrun, M., & Neumann, J. (1979). The serum-free growth of Balb/C 3T3 cells in medium supplemented with bovine colostrum. Journal of Supramolecular Structure, 11(3), 349–359. https://doi.org/10.1002/jss.400110310
Original URL	https://onlinelibrary.wiley.com/doi/epdf/10.1002/jss.400110310?saml_referrer
Source type	Journal Article
Keywords	colostrum, milk, serum, growth factors, mitogens, DNA synthesis, proliferation, 3T3 cells, serum-free growth
#Tags	# methods #analysis
Summary of key points + notes (include methodology)	Summary made by using ChatGPT Balb/c 3T3 cells respond to human breast milk by stimulating DNA synthesis, with growth-promoting factors having specific molecular weights and isoelectric points. Bovine colostrum, especially within 24 hours of birth, proves ten times more effective than serum in stimulating DNA synthesis in these cells. The reasons for this efficacy and the inactivity of milk obtained later remain unclear.
Research Question/Problem/ Need	To understand the specific factors in human and bovine milk, particularly bovine colostrum, that stimulate DNA synthesis in Balb/c 3T3 cells and the reasons for the inactivity of milk obtained after 24 hours post-birth.

Important Figures	 Fig. 3. Stimulation of DNA synthesis by bovine colostrum and by calf serum. Confluent monolayers of Balb/c 3T3 cells were incubated with various concentrations of bovine colostrum obtained on the day of the birth of a calf (○–○) and with various cocentrations of calf serum (●–●) along with (^{3}H) TdR. DNA synthesis was measured as described in Figure 1. **354:JSS Klagsbrun and Neumann**  Fig. 4. Stimulation of cell division by bovine milk. Confluent monolayers of quiescent Balb/c 3T3 cells were incubated for 6 days with various concentrations of colostrum obtained on the day of the birth of a calf (○–○) and milk obtained 10 days after birth of a calf (●–●). The final concentration of milk used were 0, 0.2, 0.4, and 0.8% (vol/vol). The cells were refed on day 3 of the experiment. On day 6 of the experiment, the cells were detached from the microtiter wells by incubation with 0.1% (wt/vol) trypsin and counted in a Coulter counter.

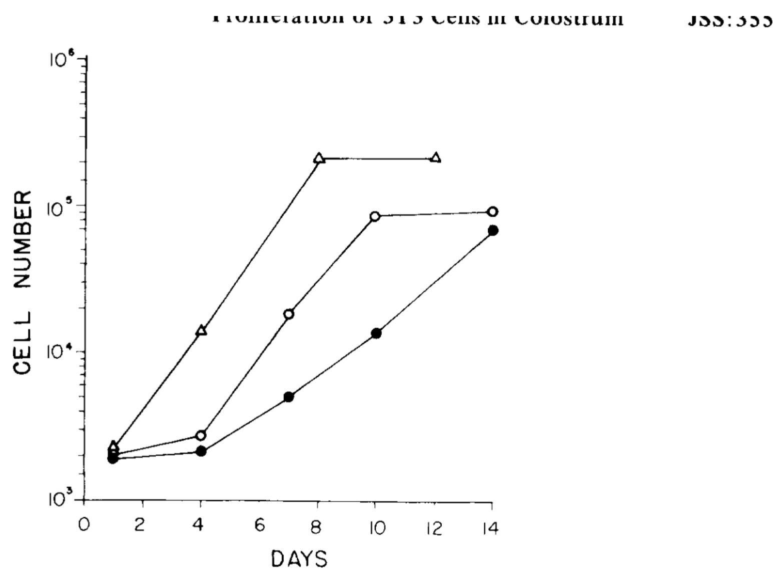


Fig. 5. The growth of Balb/c 3T3 in DMEM supplemented with either bovine colostrum or calf serum. Balb/c 3T3 cells were plated sparsely in DMEM at a density of approximately 5×10^3 cells/cm^2 and those cells that attached were grown in the appropriate medium according to the protocol described in Materials and Methods. Every third or fourth day cells in duplicate wells were trypsinized and counted while all remaining cells were refed with the approproate medium. DMEM + 10% serum (Δ–Δ); DMEM + 2.5% serum (○--○); DMEM + 2.5% bovine colostrum (●--●).

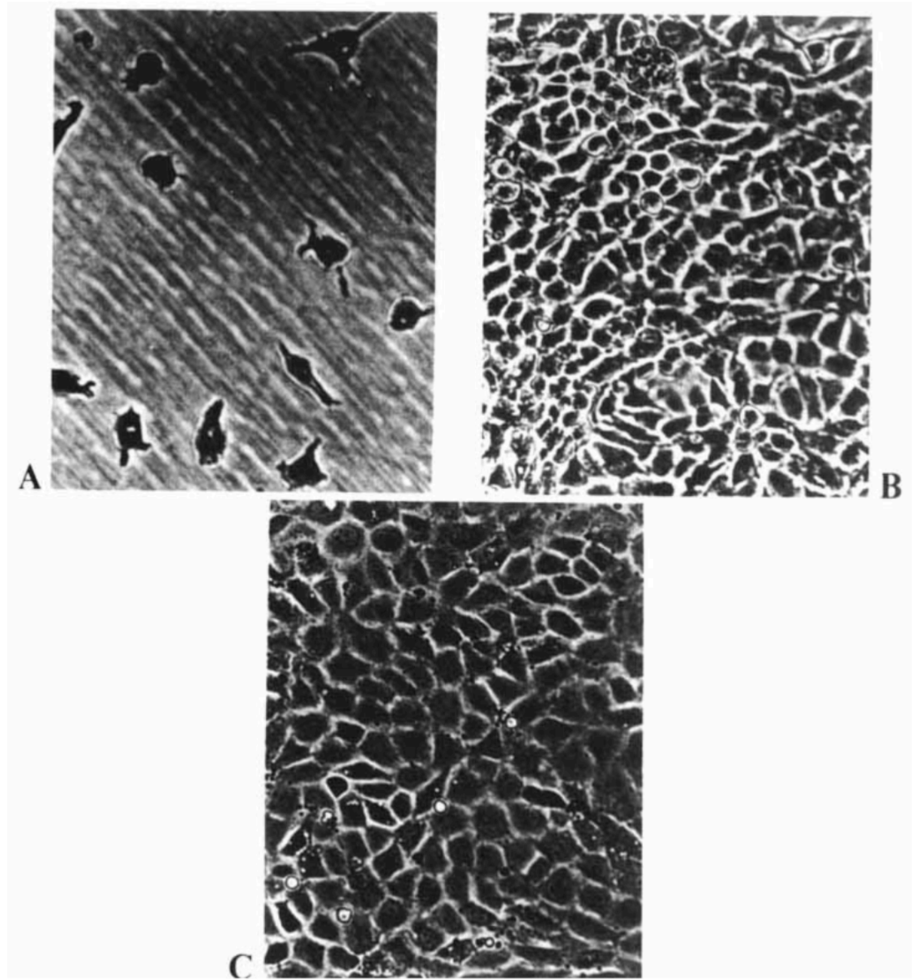


Fig. 6. Photomicrographs of Balb/c 3T3 cells grown in DMEM + colostrum or in DMEM + serum. The Balb/c 3T3 cells grown as described in Figure 5 were photographed at 1 day and 12 days after plating. A, DMEM + 2.5% colostrum 1 day after plating. B, DMEM + 2.5% colostrum 12 days after plating. C, DMEM + 10% serum 12 days after plating. The cells in 6A were photographed after fixation and staining. The cells in 6B and 6C were photographed under phase.

	352:JSS Klagsbrun and Neumann
VOCAB: (w/definition)	Isoelectric point- The isoelectric point, is the pH at which a molecule carries no net electrical charge or is electrically neutral in the statistical mean. Plating cells sparsely- cells are seeded at a low density on a culture dish, resulting in individual cells being spaced apart from each other
Cited references to follow up on	Does not provide searchable citations
Follow up Questions	Why is it that colostrum decreases in nutrient value so rapidly? What are the ethical implications of using colostrum and is it viable to use it instead of FBS in an economical point of view? What other animal colostrum can be used and which one would be the best to obtain and easiest to reproduce?

Notes:

- Balb/c 3T3 cells are a type of mouse fibroblast cells commonly used in laboratory research
- human breast milk stimulates DNA synthesis and cell division in these cells.
- molecular weights of these growth promoting factors range between 14,000 and 18,000.
- isoelectric points of these factors range between 4.4 and 4.7.
- human breast milk contains specific polypeptides that have a mitogenic activity on Balb/c 3T3 cells.
- specific molecular weights and isoelectric points of these growth promoting factors indicate their unique characteristics and potential mechanisms of action.
- typically grown in a medium supplemented with serum, which is believed to contain growth promoting factors necessary for their proliferation.
- human colostrum, the first milk produced by a woman after giving birth, had better growth factor activity compared to milk obtained later in the lactation period.
- researchers decided to try using bovine colostrum instead.

- Balb/c 3T3 cells can grow to confluence even in a medium without serum.
- Milk comes from Holstein and Jersey cows and was frozen immediately after milking

Method:

- The frozen milk samples were first thawed to bring them back to their liquid state.
- samples were spun in a centrifuge machine called RC-5 superspeed Sorvall centrifuge.
- The centrifuge machine was set to spin the samples at a speed of 12,000 X g for a duration of 30 minutes.
- separate different components of the milk based on their density.
- After the spinning process, the fat that floated on top of the spun milk was carefully removed and discarded.
- any cellular debris and other sediment that settled at the bottom of the centrifuge tube were also discarded.
- Sterilization:
  - filtration using Nalgene filter units.
  - passing the milk through a filter that has tiny pores, which can trap and remove particles or microorganism
  - the presence of casein micelles and other particles in milk can make filtration difficult, especially at higher concentrations (10% or greater).
  - samples of milk with concentrations of 10% or less were sterilized by diluting the milk into a medium (a liquid that can support the growth of microorganisms), prefiltering it with a 0.80 micron Nalgene filter unit, and then filtering it again with a 0.45 micron Nalgene filter unit.
- DMEM used in this study contained 4.5 grams of glucose per liter.
- medium was also supplemented with penicillin and streptomycin,
- Penicillin is effective against gram-positive bacteria, while streptomycin is effective against gram-negative bacteria.
- medium was also supplemented with either calf serum or bovine milk.
- Used 96 well microliter plate
- collected milk samples from cows within 24 hours after the birth of their calves.
- the milk samples obtained on the day of birth (known as colostrum) showed the highest level of activity in stimulating DNA synthesis in the Balb/c 3T3 cells.
- 1% bovine colostrum was enough to label every nucleus in the population of Balb/c 3T3 cells.
- milk samples collected after the day of birth were less effective in stimulating DNA synthesis in the Balb/c 3T3 cells.
- The concentration of bovine serum used in the experiment was 2.5% (vol/vol), which means that 2.5% of the total volume of the cell culture medium was bovine serum.
- The experiment found that bovine colostrum at a concentration of 0.25% (vol/vol) was as active as bovine serum at a concentration of 2.5% (vol/vol)
- The maximum stimulation of Balb/c 3T3 cells was obtained with 1% (vol/vol) colostrum and 10% (vol/vol) serum.
- The experiment found that colostrum was about ten times as active as serum on a per volume basis in stimulating DNA synthesis in Balb/c 3T3 cells.
- The protein concentration of bovine colostrum is about 200-250 mg/ml,

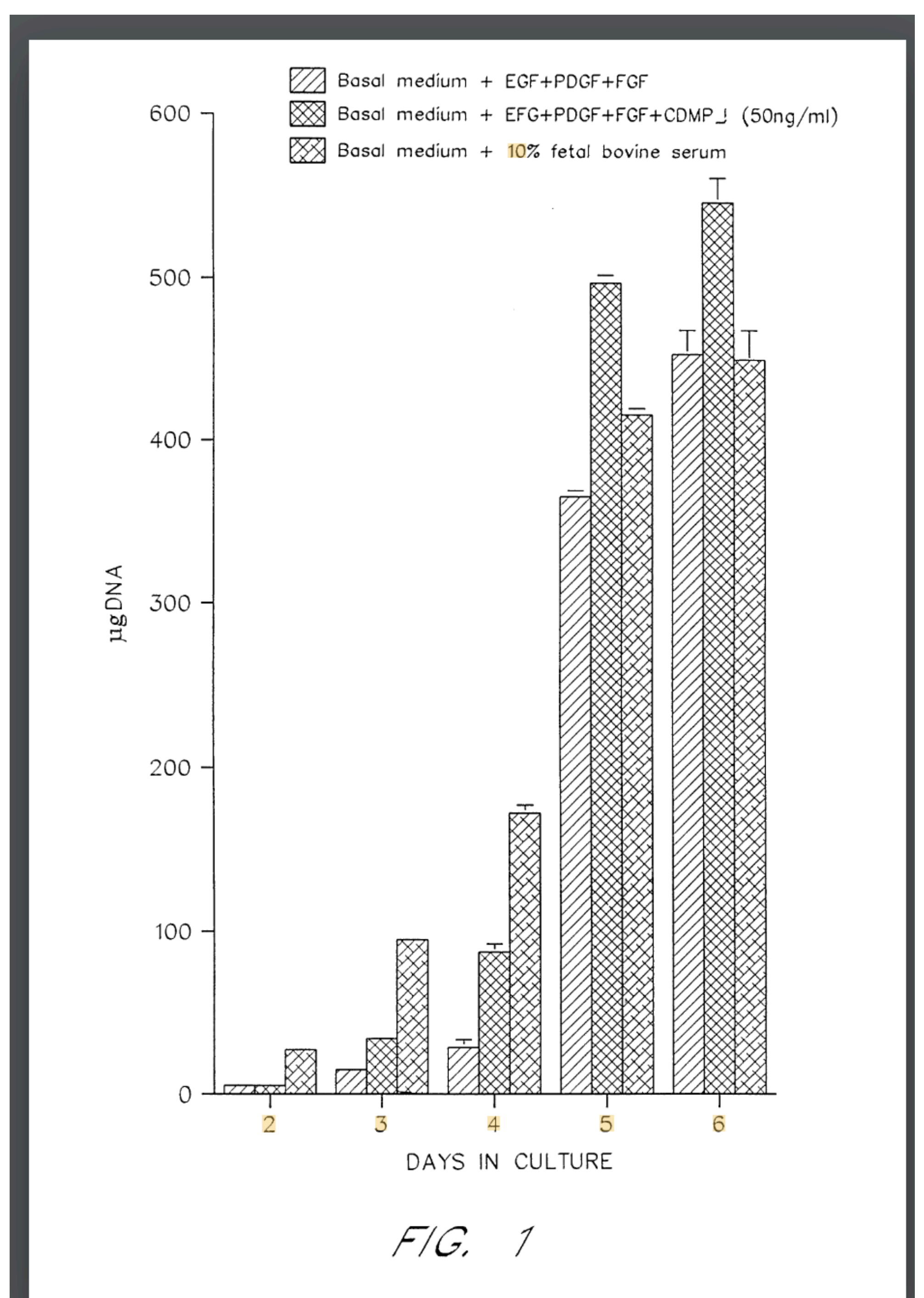
- The protein concentration of bovine serum is about 70-100 mg/ml,
- optimal concentration of bovine colostrum for the growth of Balb/c 3T3 cells was between 1% and 2.5% (volume/volume). At higher concentrations, the cells became less adhesive and detached from the dish. In comparison, the optimal concentration of bovine serum was 10% (volume/volume).
- tested the growth of Balb/c 3T3 cells in other mediums, such as milk obtained 10 days after birth and bovine serum albumin. However, there was no growth observed in these mediums.
- In serum, the cells grew uniformly throughout the dish and eventually formed a monolayer that covered the entire available surface.
- when cultured in colostrum, the cells grew in clusters and left gaps in the dish.
- clusters in colostrum were smaller and more densely packed compared to the cells grown in serum.
- In some experiments where cell loss due to lack of adhesion was minimal, Balb/c 3T3 cells grew in colostrum with a generation time equivalent to that found in serum, which was approximately 7-8 hours.
- Milk heated to 61°C for 2 hours and then cooled quickly on ice was fully active in supporting cell growth
- defatted, skimtned milk is as active as whole milk, suggesting that the growth stimulating activity of bovine colostrum resides in milk protein rather than in milk fat
- When hydrochloric acid is added to milk, it lowers the pH of the milk to 4.6.
- change in pH causes the casein protein to become insoluble and form solid particles, which is known as precipitation.
- By centrifuging the milk after the casein has precipitated, the solid casein particles are separated and removed, leaving behind the liquid supernatant.
- The supernatant fraction, or the liquid portion, has the ability to stimulate DNA synthesis in quiescent Balb/c 3T3 cells.
- The liquid portion remaining after the removal of casein, known as whey, retains the ability to stimulate DNA synthesis in quiescent cells.
- only milk obtained within 24 hours after birth of a calf, that is the colostrum, will support cell growth
- It is not clear why the milk obtained later is inactive
- cells in colostrum tend to aggregate together rather than spreading out individually.
- The lowered adhesion of colostrum-grown cells may be due to different levels of adhesion promoting factors, such as fibronectin.
- under optimal conditions, the generation time of cells grown in colostrum is approximately 28 hours, which is equivalent to that of cells grown in serum.
- The growth factors in human and bovine milk that stimulate the growth of Balb/c 3T3 cells are negatively charged polypeptides with different pH values.
- no evidence of positively charged growth factors in milk.

Patent #1 Notes: Serum-free cell growth medium

Source Title	Serum-free cell growth medium
Source citation (APA Format)	Luten, F. P. & Erlacher, L. (2001). *Serum-free cell growth medium* (US Patent US6617161B2). *Google Patents.*
Original URL	https://patents.google.com/patent/US6617161B2/en?q=(serum+free+growth+media)&oq=serum+free+growth+media
Source type	Patent
Keywords	serum-free, chondrocytes, growth medium, expansion, maintenance, phenotype, cartilage, morphogenetic proteins, CDMP-1, CDMP
#Tags	#ideas
Summary of key points + notes (include methodology)	This patent describes a serum-free cell growth medium for expanding chondrocytes. The medium contains a chemically defined mixture of nutrients and growth factors that can support the growth and maintenance of chondrocytes in vitro. The medium is effective for expanding chondrocytes from a variety of sources, including human fetal cartilage, mouse articular cartilage, and human adult bone marrow. The medium can also be used to maintain chondrocytes in a cartilaginous phenotype.
Research Question/Problem/ Need	The medium is a useful tool for researchers who are studying chondrocyte biology or who are developing therapies for cartilage repair.
Important Figures	Shows that different concentration of substances in this serum, this can be used to compare with milk

TABLE 1

Compound	Concentration
α-ketoglutarate	1×10^{-4} M
insulin	6.25 μg/ml
transferrin	6.25 μg/ml
selenium	6.25 ng/ml
bovine serum albumin	1.25 mg/ml
linoleic acid	5.35 μg/ml
ceruloplasmin	0.25 U/ml
cholesterol	
5 μg/ml	
phosphatidylethanolamine	
2 μg/ml	
α-tocopherol acid succinate	9×10^{-7} M
reduced glutathione	10 μg/ml
taurine	1.25 μg/ml
triiodothyronine	1.6×10^{-9} M
hydrocortisone	1×10^{-9} M
parathyroid hormone	5×10^{-10} M
L-ascorbic acid 2-sulfate	50 μg/ml
β-glycerophosphate	10 mM
PDGF-AB or -BB	4 ng/ml
EGF	
10 ng/ml	
bFGF	
10 ng/ml	

	 Gives data on how much dna was measured and it correlates to how many cells were produced, this can be compared with final data of my project to see if it is better
VOCAB: (w/definition)	cartilage-derived morphogenetic protein bone morphogenetic protein- signaling molecules that play a crucial role in the development and maintenance of tissues and organs.

	α-ketoglutarate-keto acid. Its carboxylate, α-ketoglutarate, is an important biological compound. It is produced by deamination of glutamate, and is an intermediate in the Krebs cycle. Ceruloplasmin- protein made in your liver. It stores and carries the mineral copper around your body. Hydrocortisone- a steroid (corticosteroid) medicine. It works by calming down your body's immune response to reduce pain, itching and swelling (inflammation). parathyroid hormone- regulates calcium levels in the blood, largely by increasing the levels when they are too low. Chondrocytes- specialized cells found in cartilage tissue,
Cited references to follow up on	None
Follow up Questions	What is the optimal ratio of the different growth factors in the serum-free cell growth medium? How will the formulation change for other types of cells? What are some similarities between the compositions of this GM, FBS and milk?

Notes:

- This cell growth medium is made up of two basal cell culture media mixed together in a 1:1 ratio (volume/volume).
- The two basal cell culture media contain certain substances that promote cell growth, including α-ketoglutarate, ceruloplasmin, cholesterol, phosphatidylethanolamine, α-tocopherol acid succinate, reduced glutathione, taurine, triiodothyronine, hydrocortisone, parathyroid hormone, L-ascorbic acid 2-sulfate, β-glycerophosphate, platelet-derived growth factor (PDGF), epidermal growth factor (EGF), and basic fibroblast growth factor (bFGF).
- 1×10^{-4} M (molar) α-ketoglutarate, 0.25 U/ml ceruloplasmin, 5 μg/ml cholesterol, and so on.
- Two basal cell culture media used in this embodiment can be selected from a group of media including Dulbecco's modified Eagle's medium (DMEM), Ham's F-12, Essential modified Eagle's medium (EMEM), and RPMI-1640.
- Medium may also contain at least one cartilage-derived morphogenetic protein (CDMP) and at least one bone morphogenetic protein (BMP).
- The method is preferably used for growing primary cells
- Another aspect of the invention involves a method for maintaining a specific characteristic (phenotype) of chondrocytes (cartilage cells) when cultured in a laboratory setting
- the method involves culturing the chondrocytes in a serum-free medium, which means that the medium does not contain any serum .
- The serum-free medium includes a cartilage-derived morphogenetic protein and/or bone morphogenetic protein, which are substances that help maintain the cartilaginous phenotype of the chondrocytes.
- The method involves several steps:
  - Isolating normal cartilage near the surface defect.
  - Isolating chondrocytes from the cartilage.
  - Culturing the chondrocytes in a serum-free medium that includes a cartilage-derived

morphogenetic protein and/or bone morphogenetic protein. This step helps expand the chondrocytes, increasing their numbers.
    - Implanting the expanded chondrocytes into the surface defect, effectively filling and repairing the damaged area.
- can be used for both short-term and long-term culturing of cells.
- growth factors include platelet-derived growth factor (PDGF)-AB or BB, epidermal growth factor (EGF), and basic fibroblast growth factor (bFGF).
- These growth factors are added to the medium to provide additional signals to the cells, promoting their growth and division.
- these concentrations can be adjusted to fit the needs of different types of cells or cell lines.
- designed for the growth of cells that require attachment to a surface (anchorage-dependent growth).
- can be used in laboratory experiments, large-scale production of recombinant proteins, and cell expansion for transplantation or implantation purposes.
- presence of type II collagen and proteoglycan aggrecan is important because regulatory agencies like the FDA/CBER require protocols for ex vivo expansion of articular chondrocytes to demonstrate that the expanded cells are similar to native chondrocytes.
- Various CDMP assays, including DNA determination, alkaline phosphatase activity, and proteoglycan biosynthesis, could only be performed in the serum-free medium.

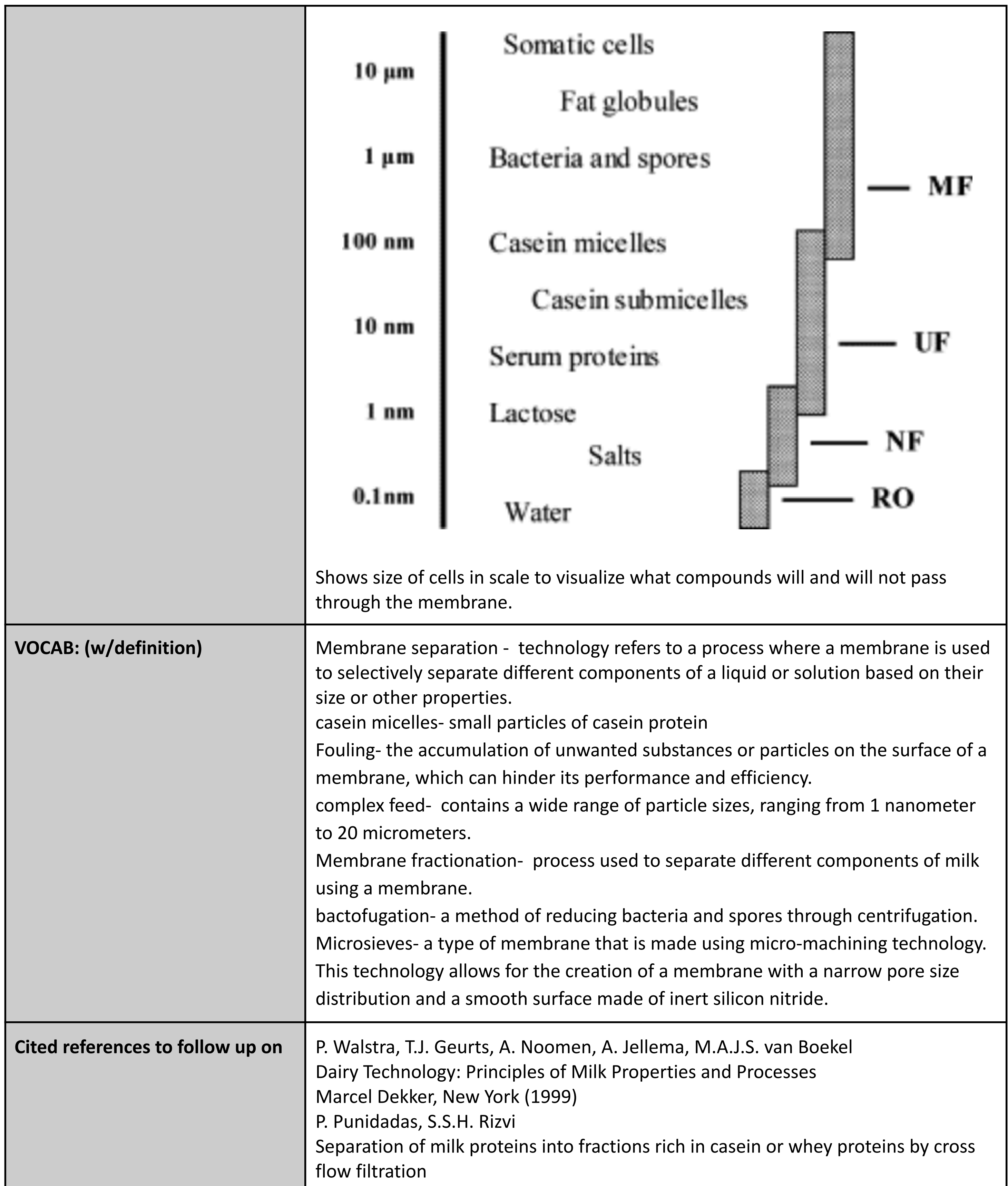
Article #17 Notes: Membrane fractionation of milk: state of the art and challenges

Source Title	Membrane fractionation of milk: state of the art and challenges
Source citation (APA Format)	Brans, G., Schroën, C. G. P. H., van der Sman, R. G. M., & Boom, R. M. (2004). Membrane fractionation of milk: State of the art and Challenges. Journal of Membrane Science, 243(1–2), 263–272. https://doi.org/10.1016/j.memsci.2004.06.029
Original URL	https://www.sciencedirect.com/science/article/pii/S0376738804004557
Source type	Journal article
Keywords	Ultrafiltration, Fouling, Milk, Whey, Protein
#Tags	#Methods #analysis
Summary of key points + notes (include methodology)	Milk fractionation, facilitated by membrane technology, involves separating its components like fat, casein, and serum proteins on a large scale. Challenges such as fouling during filtration are addressed with discussed strategies. Current industry practices focus on serum protein isolation and bacteria reduction, while future potential lies in membrane-based separation for cream and casein micelle concentration. Ongoing research emphasizes new membrane developments, module design, and computational modeling for process optimization, recognizing the complexities of milk's composition and the importance of controlling fouling for efficient fractionation processes.
Research Question/Problem/ Need	how membrane technology can be effectively utilized for large-scale milk fractionation, addressing challenges like fouling, optimizing processes, and exploring future possibilities.

Important Figures

Table 1. Average composition of cow milk: concentration and size distribution [6]

	Concentration in whole milk (g/l)	Size range and average (at weight average)
Water	87.1	
Fat globules	4.0	0.1–15 μm, average 3.4 μm
Casein (in micelles)	2.6	20–300 nm, average 110 nm
Serum proteins	0.7	3–6 nm
α Lactalbumin	0.12	14 kDa
β Lactoglobulin	0.32	18 kDa
BSA	0.04	66 kDa
Proteose-pepton	0.08	4–40 kDa
Immunoglobulins	0.08	150–900 kDa
Lactoferrin	0.01	86 kDa
Transferrin	0.01	76 kDa
Others	0.04	
Lactose	4.6	0.35 kDa
Mineral substances	0.7	
Organic acids	0.17	
Other	0.15	



	Shows size of cells in scale to visualize what compounds will and will not pass through the membrane.
VOCAB: (w/definition)	Membrane separation - technology refers to a process where a membrane is used to selectively separate different components of a liquid or solution based on their size or other properties. casein micelles- small particles of casein protein Fouling- the accumulation of unwanted substances or particles on the surface of a membrane, which can hinder its performance and efficiency. complex feed- contains a wide range of particle sizes, ranging from 1 nanometer to 20 micrometers. Membrane fractionation- process used to separate different components of milk using a membrane. bactofugation- a method of reducing bacteria and spores through centrifugation. Microsieves- a type of membrane that is made using micro-machining technology. This technology allows for the creation of a membrane with a narrow pore size distribution and a smooth surface made of inert silicon nitride.
Cited references to follow up on	P. Walstra, T.J. Geurts, A. Noomen, A. Jellema, M.A.J.S. van Boekel Dairy Technology: Principles of Milk Properties and Processes Marcel Dekker, New York (1999) P. Punidadas, S.S.H. Rizvi Separation of milk proteins into fractions rich in casein or whey proteins by cross flow filtration

	Food Res. Int., 31 (1998), p. 265
Follow up Questions	How can the methods used by the dairy industry be applied in creating a GM using diary products? How to ensure the milk is sterilized without denaturing the proteins? How to determine the behavior of cells with each protein in milk? How to adjust fro the difference in chemical composition in milk?

Notes:

- Milk is composed of various components such as milk fat, casein (a type of protein), and serum proteins.
- Separating these components into distinct fractions can allow for a more efficient utilization of their individual properties.
- Membrane processes have already been successfully employed in the dairy industry for separating serum proteins from whey and reducing bacteria and spores in skimmed milk.
- Cream separation and fractionation, which involves separating the fat-rich layer from milk, could be made possible using membrane separation.
- Concentrating casein micelles, could also be achieved through membrane separation techniques.
- strategies may include modifying the membrane surface, optimizing operating conditions, or using additives to minimize fouling.
- use of computer modeling techniques, such as analytical models and computational fluid dynamics (CFD), to gain a deeper understanding of fouling mechanisms in milk filtration.
- modeling approaches can help researchers evaluate and compare different methods for controlling fouling and optimizing various process parameters.
- Researches modeling approaches can help researchers evaluate and compare different methods for controlling fouling and optimizing various process parameters.
- Fractionating milk components allows for a more consistent quality
- Membranes are already widely used in processing whey, and their use is increasing in other dairy applications.
- Milk is a complex feed
- The concentration of dispersed components in milk is also high, at 13 weight percent. This means that there are a significant amount of different components, such as proteins, fats, and sugars, present in the milk that need to be fractionated or separated.
- The uniformity in membrane pore size is the first important factor. If the pores in the membrane are poly-dispersed (vary in size), components that should be retained may pass through the larger pores, and smaller pores may retain components that should be transmitted.
- composition of milk can vary depending on factors such as the breed, age, stage of lactation of the cow, as well as the season, climate, and feed.

Separating fat:

- in the industry, centrifugation is generally preferred over membrane separation for fat separation
- One potential advantage of membrane separation is that it can reduce energy consumption compared to centrifugation.

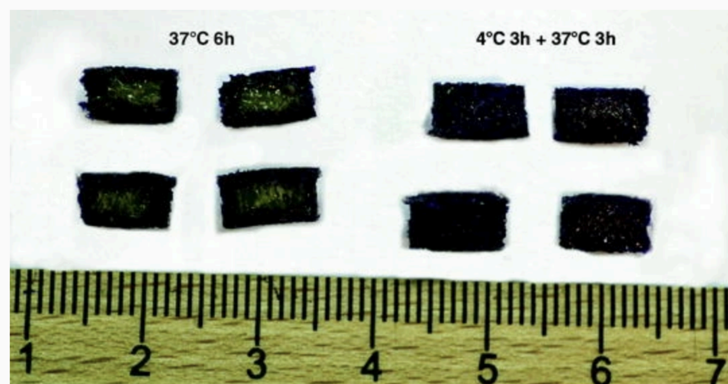
- membrane separation may cause less damage to shear sensitive components,
- The diameter of fat globules can range from 0.1 to 15 µm, with an average size of about 3.4 µm.
- At room temperature, milk fat is mostly solid. To prevent clumping of the fat globules during separation, the separation process is typically carried out at around 50 °C.
- Goudedranche and colleagues conducted a study where they used a 2 µm ceramic membrane to separate milk fat globules.
- This filtered out big fat globules but did not filter out smaller ons
- Milk was smoother but not filtered enough

Removing Bacteria

- Microfiltration (MF) is a process that can reduce the amount of bacteria and spores in milk without affecting its taste, similar to the process of Ultra High Temperature (UHT) treatment.
- Unlike pasteurization, MF provides a longer shelf life for milk.
- Saboya and Maubois conducted experiments using ceramic membranes with a pore size of 1.4 µm. The membranes were operated at a uniform transmembrane pressure of 50 kPa and a cross-flow velocity of 7.2 m/s. The flux, which measures the rate of liquid passing through the membrane, was around 1.4 × 10−4 m/s. The decimal reduction factor for bacteria and spores achieved in these experiments was above 3.5.
- Bactocatch process uses ceramic membranes and operates at high cross-flow velocities, typically 6-8 m/s.
- Filtering simulated milk ultrafiltrate (SMUF), inoculated with Bacillus subtilis, through a 0.5 µm microsieve in dead-end filtration resulted in a bacterial reduction of 6.6 decimal factors. This means that the number of bacteria was reduced by a factor of 6.6.

Article #18 Notes: Cell Viability Assays: Introduction

Source Title	Cell Viability Assays: Introduction
Source citation (APA Format)	Stoddart, M. J. (1970, January 1). Cell viability assays: Introduction. SpringerLink. https://link.springer.com/protocol/10.1007/978-1-61779-108-6_1
Original URL	https://link.springer.com/protocol/10.1007/978-1-61779-108-6_1
Source type	Protocol
Keywords	Trypan blue, RAMAN microscopy, Cell viability, Population analysis, MTT assay, LDH, Membrane integrity, cellular assays
#Tags	#methods #Analyze
Summary of key points + notes (include methodology)	Researchers use various methods to assess cell viability. The most common is the trypan blue dye exclusion assay, which distinguishes live cells (transparent under a microscope) from dead ones (appear blue). More advanced techniques like RAMAN microscopy offer detailed insights into individual cells but may require specialized equipment and expertise. The choice depends on factors like cost, speed, and complexity. Additional methods include MTT assays, live/dead staining, apoptosis studies, and LDH release assessment, each providing specific information about cell health and death processes.
Research Question/Problem/ Need	What methods can be used to determine the viability of cells and the quality of the medium?

Important Figures	Fig. 1.  The viability of whole cancellous bone cores was investigated using MTT. Freshly prepared cores were incubated for either 6 h at 37°C or for 3 h at 4°C followed by 3 h at 37°C. As can be seen by the lack of substrate conversion at the centre of the 6 h at 37°C cores, the reagent was not able to penetrate to the centre of the tissue under these conditions. This indicates that under conditions of low diffusion rates, the cells at the outer regions of the tissue can metabolise the reagent faster than the reagent can penetrate. This shows care must be taken when using metabolic labels in 3D samples.
VOCAB: (w/definition)	Colorimetry- measures the change in color Fluorimetry- measures the change in fluorescence. Provides greater sensitivity, meaning it can detect smaller amounts of cells. fluorescence at higher cell density, caution must be taken to avoid over-reduction of Alamar Blue. Live/Dead viability staining- a technique used to distinguish between live and dead cells. Calcein AM- a membrane-permeable dye that is metabolized within viable cells, resulting in cytoplasmic green fluorescence. Ethidium homodimer-1- a membrane-impermeable dye that binds to nucleic acids of membrane-compromised cells (i.e., dead cells), producing red fluorescence. Multiplexing assays- refer to a technique where multiple sets of data can be obtained from a small number of samples. Meaning multiple measurements can be made simultaneously using the same sample. Viability assays- tests that determine the number or proportion of viable cells or organisms in a sample. Necrosis- a more traumatic mechanism of cell death that occurs due to infection, toxins, or trauma.

Cited references to follow up on	Cory A.H., Owen T.C., Barltrop J.A., and Cory J.G. Use of an aqueous soluble tetrazolium/formazan assay for cell growth assays in culture. (1991) Cancer Commun. 3, 207–212.
Follow up Questions	What other types of assays exist? Any ones that dont use dyes? Are there assays to determine how well cells function instead of determining just if they are alive or not?

Notes:

- the most routine method is the trypan blue dye exclusion assay, where dead cells take up the dye and appear blue under a microscope, while live cells exclude the dye and appear transparent.
- More advanced techniques, such as RAMAN microscopy, can analyze individual cells in a highly complex manner, providing detailed information about their viability.
- The choice of assay depends on factors like cost, speed, and complexity of the equipment required.
- RAMAN microscopy may require specialized equipment and expertise, but they can provide more detailed and accurate information about cell viability.
- two types: population analysis and single-cell analysis
- single-cell analysis methods provide more detailed information about individual cells but may take longer to perform.

Trypan blue dye exclusion assay

- principle that viable cells have an intact cell membrane that can exclude the trypan blue dye.
- cells need to be in a single-cell suspension and are then visualized and counted under a microscope.
- A hemocytometer or automated counting devices can be used for this purpose.
- counting the number of viable and non-viable cells, it is possible to calculate the total number of cells and the percentage of viable cells in a population.
- these dyes can be used on cells that stick to surfaces and can be easily analyzed in large numbers.
- MTT is a commonly used dye that changes color when it is reduced by metabolically active cells. It has been widely used in research and its use in a 96-well plate format has greatly increased the capacity to analyze multiple samples simultaneously.
- in the MTT assay, the dye is converted from a soluble form to insoluble blue crystals by the metabolic activity of cells. This conversion is achieved through a reduction reaction.
- In addition to MTT, other dyes such as XTT, MTS, and WST derivatives have gained popularity in viability assays. These dyes also change color or fluorescence based on the metabolic activity of cells.
- XTT and MTS are often used in combination with a substance called phenazine methosulphate (PMS) to enhance their sensitivity. PMS helps to amplify the signal produced by the dye, making it easier to detect and measure
- Resazurin-based methods, also known as Alamar Blue, are commonly used in research.
- advantage of being able to be measured using either colorimetry or fluorimetry.
- One example of a dye combination used in live/dead staining is calcein AM with ethidium

homodimer-1.

- Live/dead staining assays can also incorporate macro-based analysis within the workflow.
- There are commercially available kits for both single assays and multiplexing.
- Earlier viability assays, such as the chromium release assay, used radioactive isotopes.
- One way to investigate apoptosis is by studying caspase activity. Caspases are enzymes that are activated during the apoptotic pathway and cleave various cell proteins as part of the programmed cell death process.
- ELISA-based methods have been developed by companies to quantify apoptosis, providing a more precise measurement.
- It plays a role in maintaining tissue homeostasis and eliminating cells that are no longer needed or have become harmful.
- Necrosis occurs due to factors such as infection, exposure to toxins, or physical trauma.
- Unlike apoptosis, necrosis is not a programmed process and often leads to inflammation and tissue damage.
- In order to determine the type of cell death, researchers can use a combination of DNA binding compounds such as SYTO probes and propidium iodide.
- th, changes occur in the integrity of the cell membrane. Healthy and early apoptotic cells have an intact cell membrane that is impermeable to DNA binding dyes like propidium iodide and 7-aminoactinomycin D (7-AAD).
- This staining can be done on a single cell level using a flow cytometer, which allows for rapid quantification of different cell populations.
- Release of lactate dehydrogenase (LDH) into culture medium is often used to assess cell death
- As the cell membrane integrity breaks down, the enzyme is released and can then be quantified.

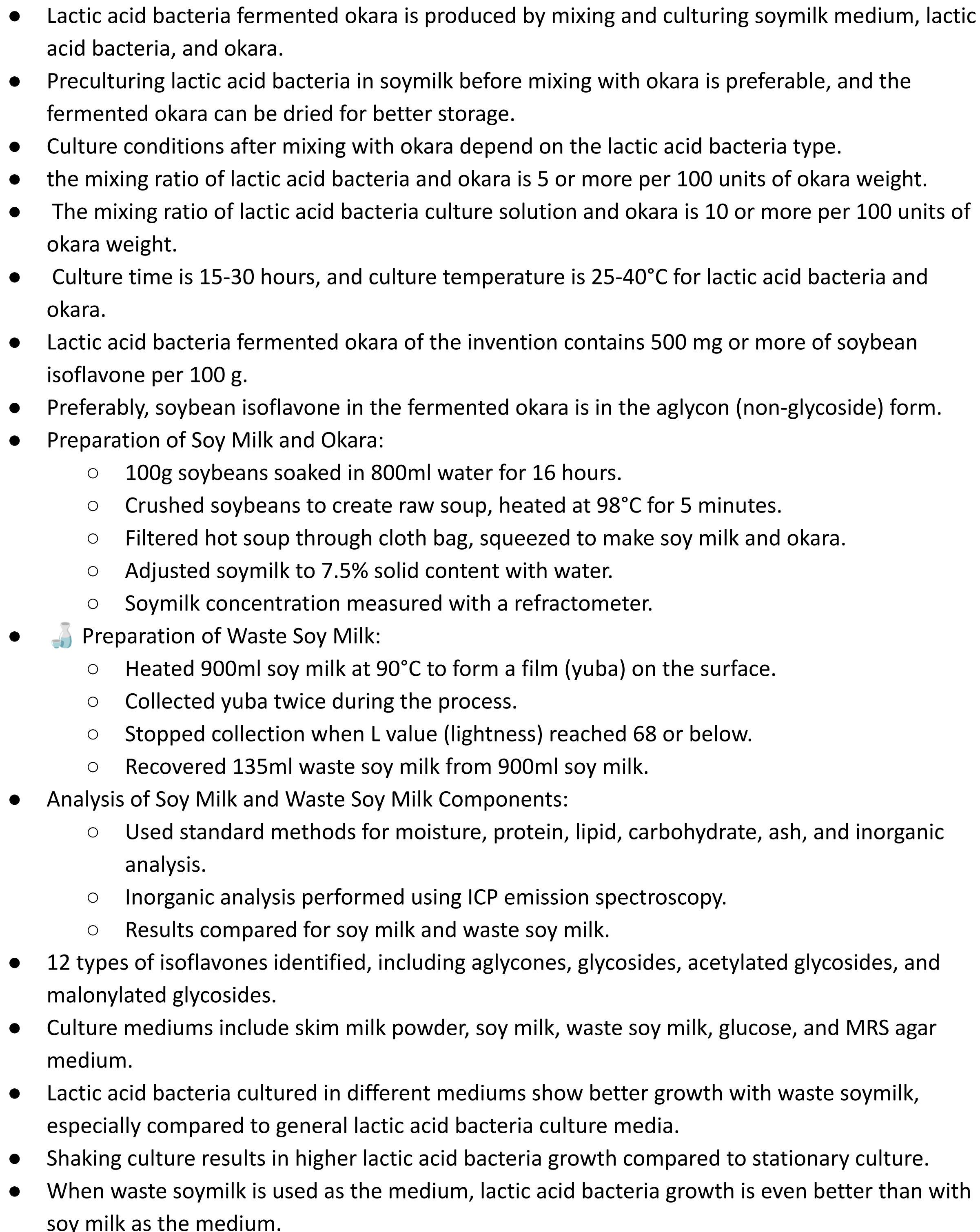
Patent #2 Notes: Okara Lactic Acid Bacteria Fermentation Using Soy Milk Medium

Source Title	Okara Lactic Acid Bacteria Fermentation Using Soy Milk Medium
Source citation (APA Format)	Kunimasa, S (2011). *Okara Lactic Acid Bacteria Fermentation Using Soy Milk Medium* (Japan Patent JP5553820B2). *Google Patents.*
Original URL	https://patents.google.com/patent/JP5553820B2/en?q=(growth+medium+okara)&oq=growth+medium+with+okara
Source type	Patent
Keywords	Okara, Lactic acid, Serum free media, Fermentation, Soy Milk
#Tags	#ideas #introduction
Summary of key points + notes (include methodology)	The invention involves a medium with soymilk (soymilk medium), a method for culturing lactic acid bacteria in the medium, and producing lactic acid bacteria fermented okara using the obtained lactic acid bacteria.
Research Question/Problem/ Need	Can lactic acid fermentation be achieved using serum free medium such as soy milk with okara supplemented?
Important Figures	None Provided
VOCAB: (w/definition)	Yuba- a film that forms on the surface of heated soy milk Soy isoflavone- found in soy, includes compounds like daidzein, genistein, and glycitein.
Cited references to follow up on	N/A
Follow up Questions	Are there potential applications of the lactic acid bacteria fermented okara in various fields like food, agriculture, cosmetics, and medicine? How can soy milk be used as a replacement for FBS? What are some similarities between soy milk and FBS, how about soy milk and bovine colostrum/milk?

Notes:

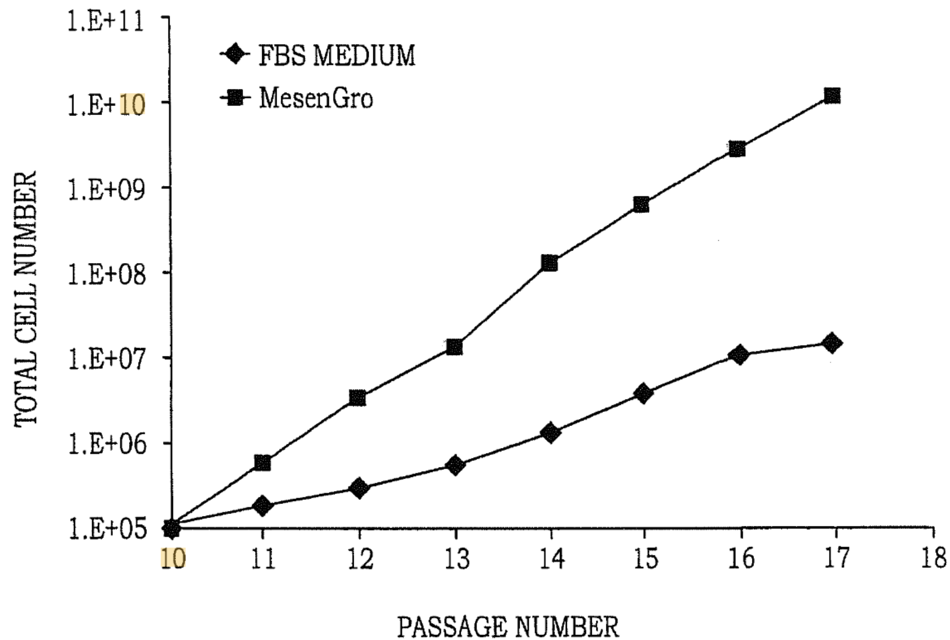
- The soymilk medium is used for a specific purpose, which is to culture lactic acid bacteria. Lactic acid bacteria are a type of bacteria that produce lactic acid as a byproduct of their metabolism.

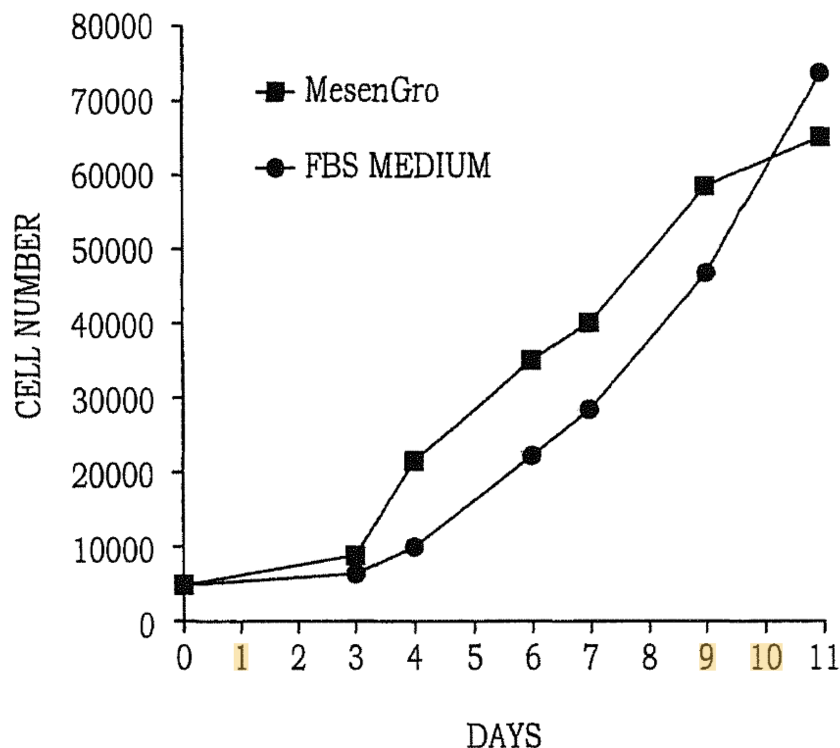
- specific strain or type of lactic acid bacteria to the soymilk medium and providing the necessary conditions, such as temperature and pH, for the bacteria to grow and ferment.
- Okara is usually discarded or used as animal feed, but in this case, it is fermented by lactic acid bacteria.
- The lactic acid bacteria ferment the okara, which likely results in changes in its composition and properties.
- found that the growth of lactic acid bacteria is significantly improved when they are cultured in a soy milk medium, specifically a brown soy milk medium obtained after collecting yuba.
- when lactic acid bacteria cultured in soy milk medium, especially brown soy milk medium obtained after collecting yuba, are used to ferment okara, the isoflavone content in the okara increases significantly.
- Soaking soybeans in water for soy milk production should be 10 hours or more, preferably 12 to 18 hours.
- Crushing methods for soybeans include using a mixer, homogenizer, or stone mortar. The rotational speed during grinding should be heat-free.
- When preparing soybean solution, the water-to-soybean ratio is 150 weights or more, preferably 180 to 200 weights.
- Heating crushed soybeans in boiling water or water at 50-100°C should take 1-10 minutes, preferably 5-8 minutes.
- Slowly agitate crushed soybeans in boiling water at a stirring speed of 10 m/sec or more, preferably 40 to 70 m/sec.
- Filtration methods for soy solution include filter cloth, sieve, or filter with hole sizes of 18 mesh or more, preferably 24 to 30 mesh.
- Soy milk can be in liquid, concentrated, or diluted form with a concentration of 6.0 to 10.0% and an L value (brightness) exceeding 70.
- Okara can be in a moist or dried form, with a water content of 7 to 15%, preferably 9 to 11%.
- Yuba, rich in vegetable protein, is formed by heating soy milk at 80-98°C for 15-40 minutes.
- Yuba production involves pulling up films formed in soy milk using bamboo skewers and continues until moisture evaporates.
- Browned soy milk has an L value of 70 or less, preferably 65 or less, due to Maillard reaction and oxidation.
- Microorganisms, including bacteria and fungi, are used, with preferred lactic acid bacteria like Lactobacillus plantarum.
- Soymilk medium can be soymilk itself, soymilk with added microbial culture components, or soymilk added to an existing microbial culture medium.
- Culture media and components for microorganisms are widely described in documents like Difco ™ & BBL ™ Manual and are commercially available.
- Components added to soymilk for microbial culture include carbon sources (e.g., glucose), nitrogen sources (e.g., ammonium salt), sulfur sources, phosphates, minerals, and vitamins.
- When adding soymilk to an existing culture medium, it should be 30% or more of the medium volume, preferably 50% or more, with a concentration of 1.8% or more in the whole medium.
- Culturing microorganisms using soymilk medium follows conventional methods, with culture conditions based on the microorganism type.

- Lactic acid bacteria fermented okara is produced by mixing and culturing soymilk medium, lactic acid bacteria, and okara.
- Preculturing lactic acid bacteria in soymilk before mixing with okara is preferable, and the fermented okara can be dried for better storage.
- Culture conditions after mixing with okara depend on the lactic acid bacteria type.
- the mixing ratio of lactic acid bacteria and okara is 5 or more per 100 units of okara weight.
- The mixing ratio of lactic acid bacteria culture solution and okara is 10 or more per 100 units of okara weight.
- Culture time is 15-30 hours, and culture temperature is 25-40°C for lactic acid bacteria and okara.
- Lactic acid bacteria fermented okara of the invention contains 500 mg or more of soybean isoflavone per 100 g.
- Preferably, soybean isoflavone in the fermented okara is in the aglycon (non-glycoside) form.
- Preparation of Soy Milk and Okara:
    - 100g soybeans soaked in 800ml water for 16 hours.
    - Crushed soybeans to create raw soup, heated at 98°C for 5 minutes.
    - Filtered hot soup through cloth bag, squeezed to make soy milk and okara.
    - Adjusted soymilk to 7.5% solid content with water.
    - Soymilk concentration measured with a refractometer.
-  Preparation of Waste Soy Milk:
    - Heated 900ml soy milk at 90°C to form a film (yuba) on the surface.
    - Collected yuba twice during the process.
    - Stopped collection when L value (lightness) reached 68 or below.
    - Recovered 135ml waste soy milk from 900ml soy milk.
- Analysis of Soy Milk and Waste Soy Milk Components:
    - Used standard methods for moisture, protein, lipid, carbohydrate, ash, and inorganic analysis.
    - Inorganic analysis performed using ICP emission spectroscopy.
    - Results compared for soy milk and waste soy milk.
- 12 types of isoflavones identified, including aglycones, glycosides, acetylated glycosides, and malonylated glycosides.
- Culture mediums include skim milk powder, soy milk, waste soy milk, glucose, and MRS agar medium.
- Lactic acid bacteria cultured in different mediums show better growth with waste soymilk, especially compared to general lactic acid bacteria culture media.
- Shaking culture results in higher lactic acid bacteria growth compared to stationary culture.
- When waste soymilk is used as the medium, lactic acid bacteria growth is even better than with soy milk as the medium.

Patent #3 Notes: Serum-free chemically defined cell culture medium

Source Title	Serum-free chemically defined cell culture medium
Source citation (APA Format)	An, s. & Zhu, Y. (2016). *Serum-free chemically defined cell culture medium* (US Patent US10287550B2). *Google Patents.*
Original URL	https://patents.google.com/patent/US10287550B2/en?q=(sereum+free+medium)&oq=sereum+free+medium
Source type	Patent
Keywords	Serum free media, STEM cells, chemically defined, FBS
#Tags	#ideas #methods #introduction
Summary of key points + notes (include methodology)	The patent application likely provides detailed descriptions of specific combinations and permutations of elements, showcasing the inventive aspects. The claims use legal language to establish the boundaries of protection. Math concepts, if present, could involve the quantitative aspects of the disclosed elements or the permutation possibilities, though the provided text doesn't explicitly mention mathematical calculations. The results would include a clear understanding of the invention's scope and potential applications.
Research Question/Problem/ Need	Creating a chemically defined media for STEM cells (and more) that are as effective as FBS and more reliable as the concentration of the compounds are defined.

Important Figures	 Fig 8) a graph of cell number over passage number which compares growth rate of adipose tissue derived MSCs (Cellular Engineering Technologies) cultured in an embodiment of the base medium supplemented with 10% FBS, L-glutamine, and penicillin streptomycin to adipose tissue derived MSCs cultured in an embodiment of the inventive serum-free medium (referred to as "MesenGro"). Fig 2) a graph of cell number over time in days compares cell growth of hMSCs in conventional medium containing 10% FBS to cell growth in the inventive serum-free medium ("MesenGro"). Cell numbers per well (24-well plate) at each time point were counted with a medium change every 2 days. Surprisingly, the graph evidences that the rate of cell growth of hMSCs in the inventive serum-free medium can be greater than in conventional medium containing 10% FBS. In certain applications this affords the advantage of allowing passage at an earlier point in time or afford the advantage of obtaining a desired number of cells in a lesser amount of time

	 FIG.2
VOCAB: (w/definition)	Non-haematopoietic- primary source of bone marrow-derived lung epithelial cells human mesenchymal stem cell-non-haematopoietic, multipotent stem cells WNT signaling pathway-group of signal transduction pathways which begin with proteins that pass signals into a cell through cell surface receptors. MCDB Medium-developed for the culture of specific cell types without a serum supplement xenobiotic proteins-chemical substances that are foreign to animal life
Cited references to follow up on	Stroma-free, serum-free, and chemically defined medium and method for ex vivo mononuclear cell expansion using the same
Follow up Questions	How did the creators determine which specific compounds to use? How similar is this to FBS, colustrum and Milk?

	How expensive are artificial media and how hard is it to gather the resources and crete them?

Notes:

- Patent discusses a serum-free chemically defined cell culture medium for culturing cells
- It includes a comparison of cell growth in this medium versus conventional medium containing fetal bovine serum (FBS)
- The medium, referred to as "MesenGro," supports similar exponential expansion of human mesenchymal stem cells (hMSCs) as conventional media
- The invention is not limited to specific components or sources, allowing for equivalent or similar elements suitable for use
- The patent application claims a broad range of embodiments, including the medium, related methods, and any variations or implicit aspects of the invention
- The description of the invention is intended to be comprehensive, including all acts facilitated by the disclosed elements
- The medium can include WNT signaling pathway activators, such as WNT3A and RSPO1, in specific concentrations
- The base medium may contain additional components like sodium bicarbonate and a regulated iron source, with specific pH adjustments[^10].
- Comparative images show cell morphology differences between hMSCs in the serum-free medium and those in conventional medium with 10% FBS
- Graphs illustrate similar growth rates of hMSCs in the serum-free medium with or without plate-coating, compared to other commercial media
- Multilineage differentiation potential of hMSCs is retained or enhanced in the serum-free medium compared to conventional medium
- Bar graphs indicate total cell numbers over different types of culturing flasks, demonstrating the medium's performance[^10].
- MSCs are crucial for skeletal repair and can differentiate into various cell types.
- Current culture media use fetal bovine serum (FBS) with limitations.
- Disadvantages of serum include potential pathogens, immune responses, and inconsistent performance.
- Invention aims for a chemically defined serum-free medium for MSCs
- Objectives include expanding MSC populations and differentiating them into chondrocytes or osteocytes.
- Various experiments and graphs demonstrate the efficacy of the serum-free medium.
- Base medium combines DMEM and MCDB in specific ratios with additional components.
- Specific concentrations for components like sodium bicarbonate, transferrin, selenium, antioxidants, steroids, and more.
- Human serum albumin (HSA) included in a particular range.
- Advantages of Culturing MSCs in Serum-Free Medium:
    - No blood-borne pathogens, such as viruses or prions.
    - No antibody generation to xenobiotic proteins.
    - Substantially lesser lot-to-lot variation.

- Compatible with various uncoated culture flasks.
- Exhibits good results in supporting MSC expansion from various sources.

Article #19 Notes: Counting cells using a hemocytometer

Source Title	Counting cells using a hemocytometer
Source citation (APA Format)	*Counting cells using a hemocytometer*. Abcam. (2023, December 13). https://www.abcam.com/protocols/counting-cells-using-a-haemocytometer
Original URL	https://www.abcam.com/protocols/counting-cells-using-a-haemocytometer
Source type	Protocol
Keywords	Hemocytometer, assay, trypan blue dye, microscope
#Tags	#methods #analyze
Summary of key points + notes (include methodology)	Protocol outlines the steps to obtain viable cell count from suspension cells using a hemocytometer in a 6:51-minute video. Aseptic technique emphasized for contamination prevention during cell suspension preparation. Hemocytometer counting process explained, including Trypan Blue treatment for viability assessment. Calculation method provided to determine viable cell concentration in the original cell suspension.
Research Question/Problem/ Need	How to use a hemocytometer to effectively count cell viability?
Important Figures	Hemocytometer diagram indicating one of the sets of 16 squares that should be used for counting.

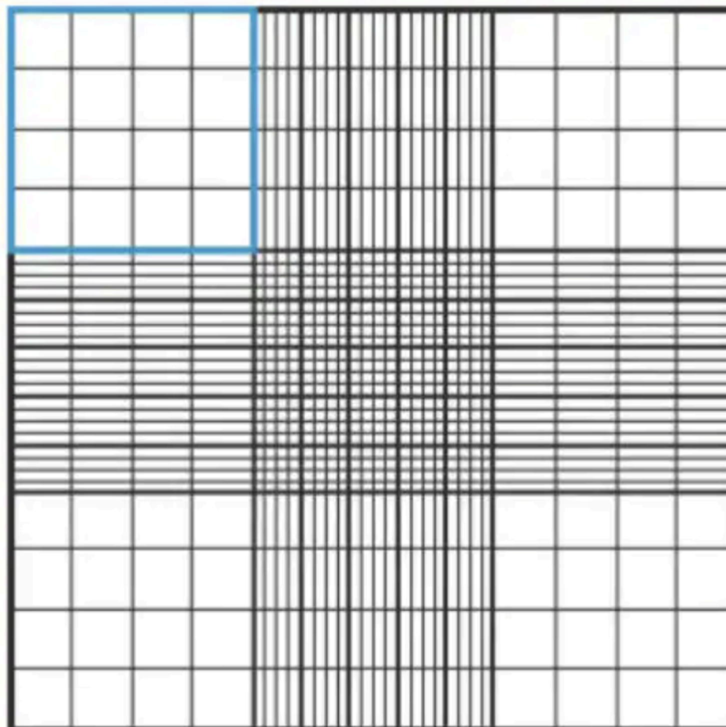


Table 1. The volume of DPBS and trypsin-EDTA required for trypsinization of adherent cells.

T-flash (cm^2)	DPBS (mL)	Trypsin-EDTA (mL)	FBS containing media required to neutralize trypsin
25	2	2	6
80	3	3	9
175	5	5	15

VOCAB: (w/definition)

Overslip- a thin, transparent piece of glass or plastic that is placed on top of the hemocytometer to hold the liquid sample in place.
Hemocytometer- a specialized glass slide that is used to count and measure the concentration of cells in a liquid sample.
Newton's refraction rings- a series of concentric circles that can be observed under the coverslip when it is properly adhered to the hemocytometer. rings are caused

	by the interaction of light with the thin layer of liquid between the coverslip and the hemocytometer. The presence of these rings indicates that the coverslip is securely attached to the hemocytometer and that there is no air trapped between them. Aseptic technique- set of practices used to prevent the contamination of cell cultures and reagents by microorganisms.
Cited references to follow up on	None Provided
Follow up Questions	How to use other dyes with the hemocytometer? What are some ways to reduce human error, are there automatic cell counting techniques? What happens is there are too many cells in the squares and they cannot be counted accurately?

Notes:

- Before using the hemocytometer and coverslip, it is important to clean them to ensure accurate and reliable results.
- Alcohol is used to clean the hemocytometer and coverslip. This helps to remove any dirt, debris, or contaminants that may be present on the surfaces.
- helps to maintain a sterile environment and prevent any interference with the cell counting process.
- it is necessary to moisten it with water.
- helps to create a thin layer of liquid between the coverslip and the hemocytometer, which allows for proper adhesion and prevents air bubbles from forming.
- ensures that the liquid sample spreads evenly across the counting chambers of the hemocytometer, allowing for accurate cell counting and measurement.
- coverslip is gently pressed down to ensure proper adhesion to the hemocytometer.
- Proper adhesion is important because it prevents the coverslip from moving or shifting during the cell counting process, which could lead to inaccurate result

Preparing cell suspension:

- take out a small amount of the cell suspension for further analysis or experimentation.
- 100 µL (microliters) of the cell suspension is taken from the Eppendorf tube and transferred to a new Eppendorf tube.
- 400 µL of a solution called 0.4% Trypan Blue is added. (or other concentrations of other dyes depending on the assay
- The contents of the Eppendorf tube are then mixed gently to ensure that the cell suspension and Trypan Blue are well combined.

Counting:

- a small volume (100 µL) of cell suspension that has been treated with Trypan Blue is taken using a pipette.
- If a glass hemocytometer is being used, the cell suspension is gently filled in both chambers underneath the coverslip. This is done in a way that allows the cell suspension to be drawn out

by capillary action.

- If a disposable hemocytometer is being used, the cell suspension is pipetted into the well of the counting chamber, and capillary action draws it inside.
- A microscope with a 10X objective is used to focus on the grid lines of the hemocytometer.
- A hand tally counter is used to count the live, unstained cells in one set of 16 squares on the hemocytometer. Live cells are those that do not take up Trypan Blue.
- cells are only counted if they are set within a square or on the right-hand or bottom boundary line.
- After counting the first set of 16 squares, the hemocytometer is moved to the next set of 16 corner squares, and the counting process is continued.
- Repeat for all 4 sets of squares

Viability

- average cell count is obtained by adding up the cell counts from each square and dividing by the number of squares (in this case, 4).
- The resulting average cell count is then multiplied by 10,000 (10^4) to account for the area of the squares and convert it to cells per mL.
- a correction factor of 5 is applied to account for a 1:5 dilution caused by the addition of Trypan Blue, a dye used to distinguish between live and dead cells.
- To check viability, you can count bot lead an live cells and find out % that died or lived

Article #20 Notes: Bovine colostrum induces the differentiation of human primary keratinocytes

Source Title	Bovine colostrum induces the differentiation of human primary eratinocytes
Source citation (APA Format)	Puppel, K., Gołębiewski, M., Grodkowski, G., Slósarz, J., Kunowska-Slósarz, M., Solarczyk, P., Łukasiewicz, M., Balcerak, M., & Przysucha, T. (2019). Composition and factors affecting quality of Bovine Colostrum: A Review. *Animals*, *9*(12), 1070. https://doi.org/10.3390/ani9121070
Original URL	https://www.mdpi.com/2076-2615/9/12/1070
Source type	Journal Article
Keywords	Bovine colostrum, growth factors, calf, calving; quality, immunoglobulin, mammary gland, colostrum
#Tags	#methods #analysis #ideas
Summary of key points + notes (include methodology)	In an effort to enhance dairy cow production traits, the importance of calf rearing and colostrum quality is emphasized for improved profitability. Colostrum, produced in the final stage of pregnancy and early post-calving, plays a crucial role in providing necessary nutrients and immune components to calves. The first 2 weeks are critical for calf health, with disorders potentially leading to poor colostrum quality and overall husbandry issues.
Research Question/Problem/ Need	The article explores the composition and factors influencing the quality of bovine colostrum, presenting data on mineral, chemical, and amino acid content over time.

Important Figures

Table 6. The amino acid composition of colostrum and milk (g/kg) [5].

Amino Acids	Colostrum	Milk
Aspartic Acid	42.95	28.83
Threonine	33.26	14.64
Serine	44.95	19.74
Glutamic Acid	88.84	91.12
Proline	25.96	56.98
Cystine	8.51	2.39
Glycine	15.65	5.96
Alanine	15.79	11.42
Valine	28.33	16.95
Methionine	9.31	12.00
Isoleucine	15.1	13.67
Leucine	47.30	35.94
Tyrosine	39.56	15.34
Phenylalanine	25.22	17.16
Histidine	14.60	12.12
Lysine	40.90	28.51
Arginine	14.40	10.22

Table 1. Changes occurring in the chemical composition of colostrum from the first collection depending on the species of farm animals [5].

Species	Chemical Composition (g/kg)		
	Fat	Protein	Lactose
Horse	7	191	46
Cattle	36	130	31
Sheep	124	130	34
Goat	90	80	25
Pig	72	180	24
Dog	78	138	27
Rabbit	47	135	16

VOCAB: (w/definition)	Radial immunodiffusion-RID is an older method based on the classic precipitin reaction in which antigen and antibodies react to form precipitates in liquid or semi-fluid media. Under conditions of antibody excess, the quantity of the precipitate is directly related to the quantity of antigen in the test sample. Refractometer-A refractometer is a laboratory or field device for the measurement of an index of refraction.
Cited references to follow up on	None
Follow up Questions	How do these changes in colostrum and milk composition impact the nutritional value for calves? Are there specific factors that affect the quality of bovine colostrum besides the time from calving? What can be concluded as the optimal time after berth to collect colostrum from a mother.

Notes:

- Introduction:
  - Colostrum is vital for immunity and cell-mediated immunity during the perinatal period.
  - Chemical composition varies among animals, offering immunoglobulins, growth factors, and antimicrobial substances.
- Colostrum's Chemical Composition
  - Composition changes over time; immediate feeding is crucial for optimal absorption of immunoglobulins.
  - Immunoglobulins (IgG, IgM, IgA) provide passive immunity, and their absorption decreases over time after birth.
  - Colostrum includes proteins, lysozyme, lactoferrin, lactoperoxidase, lactose, and minerals essential for calf development.
- Colostrum's Quality Evaluation
  - Timely feeding of colostrum is essential, with the first 2-4 hours post-birth being crucial for immunoglobulin absorption.
  - Failure of passive transfer of immunity can lead to economic losses, including calf mortality and morbidity.
  - Colostrum provides vital nutrients such as fat, protein, vitamins (A, D, E), and minerals for calf growth and immune support.- Feeding Methods:
  - Tube-fed calves consume more colostrum in their first meal than bottle-fed calves.
  - Nipple bottle feeding increases serum Ig concentrations when calves are fed 1.5 L of colostrum.
- Evaluating Ig Levels:

- Radial immunodiffusion and colostrometer are standard methods for Ig evaluation in colostrum.
- Colostrum density correlates with Ig content; colostrometer measures density and indicates quality levels.

- Measurement Devices:
  - Refractometer is more precise than colostrometer, requires a small colostrum sample, and has high correlation with Ig concentration.
  - RID requires a specialist lab, takes time, and has lower availability.
- Factors Affecting Colostrum Quality:
  - Calves need 100–200 g of Ig within 6 hours for good to very good colostrum quality.
  - Quality varies due to factors like parity, pre-partum diet, season, breed, and dry period length.
- Composition of Colostrum:
  - Colostrum differs from milk in physicochemical properties and ingredient proportions.
  - Contains more active compounds, proteins, minerals, vitamins, and fewer carbohydrates and lipids.
- Genetic Factors:
  - Heritability affects immunoglobulin concentration; breed influences colostrum quality.
  - Difficulty in birth, diseases, and age of the cow impact colostrum properties.
- Storage Methods:
  - Storing colostrum in open vessels at warmer temperatures alters bacteria count and pH.
  - Chemical additives and bacterial fermentation can extend the storing period.
- Preservation Methods:
  - Heat treatment reduces bacterial levels but may decrease Ig concentration.
  - Freezing at -18 to -20 °C preserves colostrum for up to six months.