https://doi.org/ 10.33472/AFJBS.6.Si2.2024.1727-1739

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

In Vitro Meat Production: Advances, Challenges and Future Perspectives

Carlos Jácome-Pilco[1];
0009-0008-2092-9577;
Anderson Eliceo-Manobanda[1];
0009-0007-4766-0355;
Pedro Elehan-Vistin[1]
0009-0008-1895-4650
[1]Universidad Estatal de Bolívar, Facultad de ciencias agropecuarias, recursos naturales y del medio ambiente, Ingieneria Agroindustrial, Laguacoto II, via a la costa Kilometro 6

Article History
Volume 6,Issue Si2, 2024
Received:13 Mar 2024
Accepted : 16 Apr 2024
doi: 10.33472/AFJBS.6.Si2.2024.1727-1739

Abstract

The research addresses the growing demand and challenges linked to sustainable meat production in the current context of population growth and concerns for animal welfare. It highlights the nutritional relevance of meat and the pressure on natural resources derived from conventional animal husbandry. Laboratory-grown meat is introduced as an alternative, exploring its production through tissue engineering and cell culture techniques, highlighting its potential to reduce dependence on mass livestock farming and its environmental benefits. The methodology, qualitative and based on systematic reviews, focuses on advances, challenges and future perspectives. Large-scale production is detailed, addressing aspects such as cell culture, identification of suitable cells, culture media formulation, and energy efficiency. The research highlights the importance of lab-grown meat as a promising option to address challenges in the meat industry. This type of meat, whose cellular scaffold is commonly based on porous hydrogels and non-animal materials, uses bioreactors of up to 50,000 liters to ensure optimal conditions. The nutritional and sensory aspects demand the incorporation of essential nutrients and strategies to improve color and flavor, facing challenges in the imitation of processed meat products. Ethics, costs, legal and religious considerations, as well as production limitations, constitute barriers. Despite the challenges, cultured meat offers a sustainable solution for food security and reduced environmental impact. **Keywords:** In vitro meat, cell culture, bioreactor, cultured meat, cultured meat

1. INTRODUCTION

Due to the nutritional relevance and continued popularity of meat as a food, as well as the increase in the world's population, the meat industry has experienced considerable growth, this exponential growth poses a monumental challenge to the sustainability of the meat breeding system (Pol, 2021), in addition to the focus on animal welfare has become increasingly important, especially when it comes to animals that are raised on farms. Every year, around 1380,000 million animals are slaughtered, and of these, 3,800 million are used for daily food (Gisie, 2022).

Lab-grown meat is a new technology that has similar characteristics compared to conventional meat. Instead of raising and slaughtering animals for meat, this technology relies on growing edible muscle tissue from muscle cells or stem cells; it seeks to imitate the structure and flavor of traditional meat using tissue engineering techniques in a controlled culture environment (Burgos, 2023).

Cell culture is a technique used in in vitro studies to maintain and analyze cells outside the body, this method involves the cultivation of cells from an organ or tissue in controlled media with the purpose of increasing their number and inducing their differentiation (Cartín-Rojas & Ortiz, 2018). Likewise, cellular bioreactors are used for meat production in laboratories, thus avoiding the slaughter of animals, improving energy efficiency and the use of resources in meat production. It is anticipated that in the future, conventional farms could be replaced by companies specializing in the production of cultured meat (Párraga, 2022). Based on the above, the main objective of this research is to explore the advances, challenges and challenges of and future prospects in in vitro meat production. disseminated from different investigations carried out.

2. Obtaining Lab-Grown Meat

Tissue engineering techniques can be employed to produce meat in vitro by expanding cells obtained from muscle tissue samples (Jones et al., 2021), growing muscle cells that develop into new tissue in specific liquid media, the differentiation phase forming myotubes and a muscle fiber within three weeks (Ismail et al., 2020).

This procedure offers the potential to decrease reliance on large herds of livestock, which in turn could contribute to the reduction of greenhouse gas emissions and agricultural land use (Jones et al., 2021). This potentially safer and healthier cultured meat eliminates allergenic substances present in plant-based meat, but fetal bovine serum and antibiotics need to be eliminated for sustainability and acceptance (Ismail et al., 2020).

Lab-grown meat products are designed with the goal of replicating the sensory characteristics, such as appearance, texture, taste, smell, and nutritional value of traditional meat. These products were first accessible to consumers in Singapore at the end of 2020, while the inauguration of the first commercial factory took place in Israel in 2021, with a production capacity of 500 kg of meat per day (de Oliveira et al., 2022).

2.1 Cell cultures

The methods used in the production of in vitro meat involve the cultivation of muscle tissue in a culture medium or in a bioreactor, where rigorously controlled parameters are applied. This process is based on the principles of regeneration used in human medicine to rebuild impaired muscle tissue in patients by using their own cells, serving as inspiration for the creation of artificial meat (Bailone et al., 2019).

Cell culture involves processing a tissue sample to extract and place cells in a feeding medium, providing environmental conditions conducive to their survival and cell division. These cultured cells can specialize into different cell types through differentiation, allowing them to perform functions similar to those they would have in various tissues or organs of the body (Morales-Sánchez et al., 2019).

Based on the above, the production of cultured meat involves the cultivation of animal cells in a bioreactor that requires sterility and controls variables such as temperature and pH, in this environment, the cells multiply and specialize, forming, for example, muscle tissue. Researchers are currently working on specialized bioreactors for the large-scale production of products derived from cellular agriculture (Gisie, 2022).

2.2 Process for obtaining lab-grown meat

In vitro meat is characterized as lab-grown meat in a bioreactor under artificially controlled conditions (Moyano, 2021). To generate one kilogram of meat in this environment requires the production of approximately 50 billion cells outside the body (ex vivo). Subsequently, the formation of muscle fibers is replicated in vitro, which are harvested and processed (Gauna & Pérez, 2018).

Among the cell types crucial for industrial production are muscle satellite cells, myoblasts, myocytes, adipose-derived stem cells (ADSCs), adipocytes, and fibroblasts. These cells represent a variety of muscle cells in an order ranging from the most similar to the most Differentiated. ADSC Precursors Are Fat Cells That May Be Helpful
for the production of the fatty component of cultured meat products, while fibroblasts, connective tissue cells, contribute to the texture and structure of the products (Marthen, 2021).

In the cultured meat production process, muscle stem cells are extracted by muscle biopsy from a donor organism and grown in the laboratory by in vitro muscle proliferation (Woll, 2018), by using digestive enzymes such as p. trypsin or collagenase to release cells from muscle samples (FAO & WHO, 2023). These cells develop into growth media that promote their multiplication. Subsequently, stem cells undergo a process of differentiation, transforming into muscle cells and fibers (Woll, 2018).

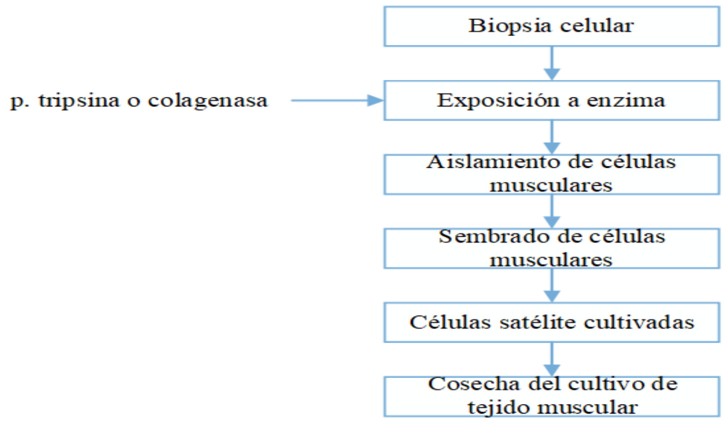


Figure 1

Flowchart for the sourcing and isolation of cultured muscle satellite cells. In original language: Spanish

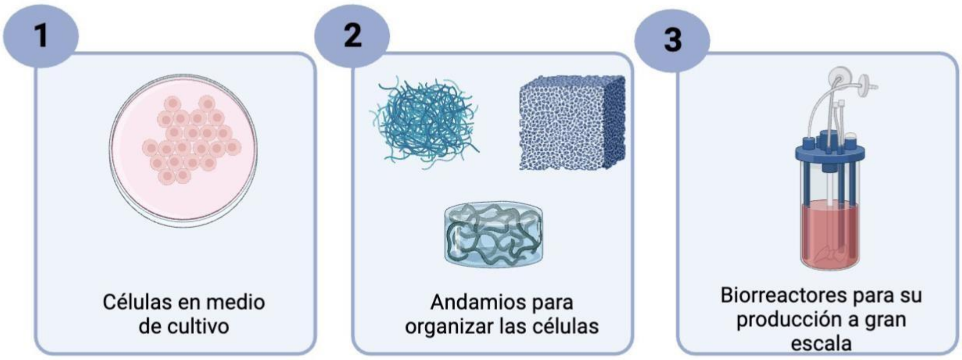
Source: (FAO & WHO, 2023).

The expansion of muscle cell culture is carried out in large-scale industrial bioreactors, in which muscle stoles are collected and processed, adding various compounds to improve nutritional value, color, flavor and texture. To achieve the production of a specific cut of meat, it is used additional technology aimed at instituting muscle cells into the proper structure and shape (Zuñiga et al., 2021).

To produce this type of "meat" it is necessary to consider the identification of appropriate cells, the search for a suitable growth medium, not of non-animal origin to avoid the risks of communicable diseases, the obtaining of suitable and edible materials for the matrices along which, to produce denser pieces of meat, as well as the development of efficient bioreactors (Marthen, 2021).

Figure 2

Graphical representation of the essential phases in the production of synthetic meat. In original language: Spanish



(1) Cell culture in in vitro environments. (2) Use of scaffolds for cell organization and assembly. (3) Scaling up to industrial levels through the use of bioreactors. Source: (FAO & WHO, 2023).

2.3 Culture Media and Bioreactors Used for Laboratory Meat Production

Culture media play a role in consistently supplying the necessary nutrients to proliferating cells. They are prepared from fetal bovine sources, newborn or adult. However, these media are expensive and have a variable composition depending on the source, making them unsuitable for large-scale production at the industrial level (Kumar et al., 2021).

Whey free media made from plant or synthetic compounds have been developed that provide growth potential comparable to that of whey media. An example is the plant media rich in amino acids obtained from the mushroom extract maitake, which have been identified as optimal for promoting the growth and expansion of the surface area of fish explants. (Stephens et al., 2018). New culture media incorporating supplemental proteins such as AIM-V, Sericin and Ultroser G are also presented. AIM-V media were observed to increase active tension during cell differentiation (Kumar et al., 2021).

Also, cyanobacteria are widely recognized as a promising source of energy and supplements for cell growth. Their cultivation is highly accessible and they contain a remarkable amount of protein, accounting for 70% of their dry matter (Post et al., 2020).

For (Risner et al., 2020) in the production of laboratory-cultured meat, an inoculum with a cell concentration of 2.00×10^{8} cells/ml is used, in an inoculum bioreactor volume of 2.00 L, where the desired mass of meat produced is 1.21 × 108 kg, where the cell doubling time is 8 hours, and the glucose and oxygen consumption rates per cell are 4.13×10^{-14} Mol/H cell and 1.80 × 10−14 Mol/H cell, respectively. The energy efficiency of the boiler is 85%, with 50% self-generated electricity. The temperature of the medium entering the facility is 20°C, while the desired temperature of the medium entering the bioreactor is 37°C. These parameters are essential for the efficient production process of lab-grown meat.Various antibiotics such as amphotericin, fungizone, gentamicin, penicillin, streptomycin, or antimitotics need to be incorporated into cell culture media to control microbial contamination. Compounds such as dexamethasone and cell signaling molecules (heparin sulfate, hepatocyte growth factor, insulin-like growth factor, interleukin, beta-catenin signaling inhibitor, etc.) are also added. (Choi et al., 2021)

2.4 Scaffolding/Cellular Substrate

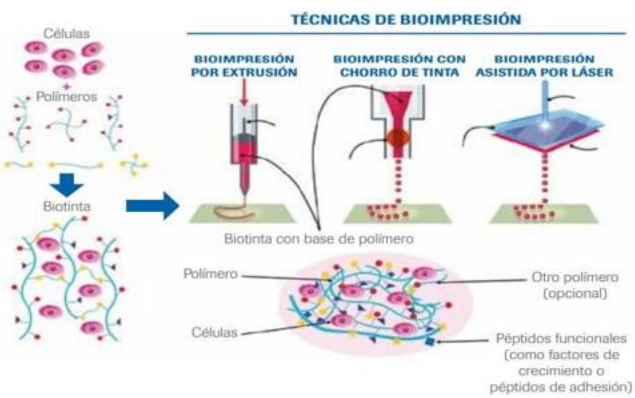
Efficient cell culture requires adequate support to obtain a tissue-like structure, providing an optimal environment for cell growth, similar to the extracellular matrix in vivo (Post et al., 2020). A solid scaffold is essential for the growth and adhesion of myoblasts, maximizing their surface area and facilitating their anchorage. During in vitro meat production, the scaffold must ensure efficient fixation and optimal nutrient use (Ben-Arye et al., 2020). Today, porous hydrogels are commonly used as the basis for cell scaffolding, ensuring adequate access of nutrients and oxygen to cells (Kumar et al., 2021).The ideal scaffold should come from a non-animal source, such as collagen alternatives, have a wide surface area for cell growth and adhesion, be flexible with tissue-like rigidity for proper myotube development, allow for optimal diffusion of the medium, and be easily separable from meat cultures (Post et al., 2020). Various edible and inedible polymers, such as collagen, cellulose, and synthetic polymers such as poly(L)lactic acid, have been suggested as base materials for scaffolding. In addition, structures from textured soy protein have been developed for the production of cultured meat. By fabricating a gel-like base material mimicking original tissues, decellularized extracellular matrix bioinks are incorporated along with tissue-specific growth factors and adhesive proteins to enhance cell growth and differentiation (Kim et al., 2020).

Figure 3

Main Bioprinting Techniques. In original language: Spanish

In the manufacture of a base material that mimics original tissues, a similar gel is used and extracellular matrix bioinks are incorporated, along with tissue-specific growth factors and adhesive proteins.

Source: (Jorcano, 2019).



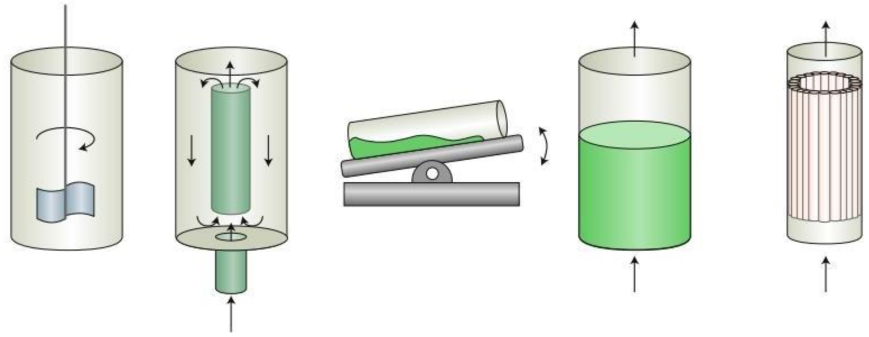
2.5 Bioreactor

Bioreactors used in in vitro meat production facilitate biological and biochemical reactions under carefully controlled environmental and operational conditions, such as pressure, temperature, oxygen supply, waste removal, and pH. This ensures a high level

of accuracy, reproducibility, repeatability, and automation required for large-scale operations (Kumar et al., 2021).
For the commercial production of cultured meat, it is essential to have bioreactors of sufficient size and enclosed space to allow for the proper growth and differentiation of the large number of cells needed to commercialize this technology (Suresh, 2018). The U.S. company Eat Just used a 10,000-liter bioreactor to prepare chicken bites in Singapore, proposing 50,000-liter bioreactors for the global marketing of in vitro meat (Kim et al., 2020).
In the case of rotating wall vessel bioreactors, the medium flows in a laminar flow by a rotating wall that maintains the proper balance between centrifugal, gravitational, and drag force. This balance is critical to achieve optimal diffusion with a high mass transfer rate and low stress of
cut for growing cells, immersed in the medium in three-dimensional free-flowing states (Allan et al., 2019). In the context of scaffold-based cell culture technology, direct diffusion bioreactors are suggested due to the adequate flow of growth media and gas exchange through porous scaffolds (Kumar et al., 2021).

Figure 4

Bioreactors Most Frequently Used in Mammalian Cell Culture



The bioreactors most frequently used in mammalian cell culture are represented in the order from left to right: stirred tank, airlift, roll/wave, fluidized bed or fixed/packed bed, and hollow fiber.

Source: (Post et al., 2020).

3. Nutritional and Sensory Aspects of Cultured Meat

One of the main purposes of meat substitutions is to ensure that they have a nutritional value comparable to that of conventional meat, however, deficiencies are observed in certain components, such as vitamin B12, which is usually obtained from the environment (Cuevas, 2020). Therefore, to improve nutritional quality, it is imperative to incorporate iron and vitamin B12 into this cellular product. In addition, it is necessary to add both natural and synthetic growth hormone to promote their proper development. (Llano, 2021).
It is essential to fortify cultured meat with the same nutrients present in conventional meats to ensure equivalent nutritional value. Amino acids such as creatine and taurine stand out, vital for muscle energy and metabolic processes (Fraeye et al., 2020). B vitamins are crucial for the

nervous system and red blood cell production. Micronutrients such as iron, essential for hemoglobin and oxygen transport. These are key examples of nutrients needed in cultured meat (Soucase, 2021).

3.1 Sensory aspects

Regarding the sensory aspects of cultured meat, which has a grayish color due to the absence of myoglobin under oxygen conditions, to counteract this, the introduction of myoglobin through hypoxia conditions, direct addition to the medium or iron supplementation is investigated (Fraeye et al., 2020). In order to emulate the taste of meat products such as hamburgers or sausages, emulsions that encapsulate fats or the addition of flavor-enhancing molecules can be applied, in essence, the use of additives used in the industrial production of meat products is resorted to (Tomiyama et al., 2020).

Creating synthetic meat that mimics meat pieces faces challenges, such as the lack of

of a vascular system. To achieve larger pieces, a similar vascular system and the inclusion of additional cells are needed. Although mimicking processed meat products is more feasible, the texture often resembles that of industrially processed products rather than fresh meat (Fraeye et al., 2020). The Israeli company Aleph Farms has advanced with 3D bioprinting to create a fillet prototype, but they are looking to reduce costs and increase scalability (Soucase, 2021).

4. Present Challenges for Cultured Meat Production

4.1 Ethics in development and application

Cultured meat seeks to reduce animal cruelty linked to the livestock industry, making it a significant option for those advocating for animal rights (Reyes et al., 2024). Unfortunately, fetal bovine serum (BFS) is used as a reagent to deliver growth factors.

obtained through the extraction of blood from cattle by cardiac or umbilical puncture, resulting in the death of the mother and fetus (Chriki & Hocquette, 2020).

Another relevant aspect is production costs, although these have decreased to become more affordable, synthetic meat continues to be perceived as a luxury item due to its high selling price (Reyes et al., 2024). In this context, the cost per kilogram is \$80, while conventional meat is marketed at \$5.6 (Gaydhane et al., 2018). Although lab-grown meat appeals to those looking to reduce its consumption, a global research conducted in 2020 with 6,128 participants revealed resistance to consuming synthetic meat, as they consider it unnatural and question the ethics of its production (Zuñiga et al., 2021).

4.2 Legal and Religious Considerations

Although this type of meat is not yet on the market, regulations require Good Manufacturing Practices (GMP) and Hazard Analysis and Critical Control Points (HACCP) certifications for marketing, along with regular government inspections. This ensures that the consumer has access to safe food that meets quality standards (FAO & WHO, 2023). Likewise, with regard to labeling, the Ministry of Health stresses the importance of including information such as the cell line used, the percentage of cells with respect to the total product, terms established by the regulatory body, and the elements required in conventional labeling (Reyes et al., 2024).

In Jewish and Islamic traditions, synthetic meat must meet Kosher and Halal standards, which involves avoiding animal suffering. In 2022, some rabbis considered synthetic meat derived from bovine embryonic stem cells to be "Kosher Parve," meaning that it could be consumed (FAO & WHO, 2023).

4.3 Limitation in the production of synthetic meat

The production of synthetic meat faces significant challenges, with the high initial investment for its large-scale commercialization being the main constraint on competition with conventional meat (Tibrewal et al., 2023). This challenge is mainly attributed to Fetal Bovine Serum (BFS), which can account for between 55% and 95% of production costs, depending on the amount used. From a societal perspective, concerns arise related to the perception of taste, texture, and safety, compounded by persistent concerns about "unnaturalness" (Gómez, 2021).

In addition, the current production in specialized laboratories in small quantities

raises the need for a transition towards industrialisation, which entails technical and logistical challenges, given that culture media must guide cell differentiation to form muscle tissue, it is essential to establish a sterile and controlled culture environment; accurate reproduction of the taste and texture of conventional meat is also presented as a significant obstacle (Reyes et al., 2024). The specific profiles that make meat attractive don't come from a single component, but of one Complex combination of molecules, including amino acids, fats, sugars, and volatile compounds, as well as the fibrous structure and water-holding characteristics present in animal meat (Reyes et al., 2024).

5. Economic and Trade Outlook

The future of synthetic meat looks talented and anticipates considerable growth in the coming years. Based on projections, cultured meat is expected to replace its conventional counterpart, as its competitive advantages will outweigh the problems associated with the presence of toxins, antibiotics, and hormones (Tibrewal et al., 2023). With the advancement of technology and the decrease in the costs of production, synthetic meat is projected to become more affordable and common in the market. Significant investments are being made in research and development with the aim of improving production efficiency, increasing scale, and perfecting its sensory characteristics (Reyes et al., 2024). As has become evident, traditional meat production has a devastating impact on the environment, fortunately, synthetic meat promises to reduce these problems by using fewer natural resources. However, challenges remain, such as the use of Fetal Bovine Serum (FBS), so it is imperative to develop a substitute that provides the necessary nutrients for synthetic meat as soon as possible (Gisie, 2022). With more companies entering the synthetic meat market and establishing appropriate regulations, it is highly likely that a greater variety of products will be introduced (Reyes et al., 2024).

6. Sustainability & Environment

The raising of animals for consumption represents a highly polluting sector, with livestock contributing 14.5% of greenhouse gas emissions (Gisie, 2022). In particular, cattle play a considerable role in global greenhouse gas emissions, mainly due to the release of methane (CH_4) into the digestive systems of herbivores, in addition to carbon dioxide (CO_2) and nitrous oxide (N_2O). In contrast, the emission associated with cultured meat is CO_2, caused by the use of fossil energy to heat cultured cells. (Chriki & Hocquette, 2020).

Therefore, artificial meat could present a more sustainable alternative in terms of greenhouse gas emissions, as it demands less land, water and feed compared to conventional livestock farming. In addition, by eliminating the need to raise animals, deforestation and deforestation are significantly reduced.

water pollution associated with intensive animal husbandry (Dantas & Pinto, 2022).

Likewise, cultured meat is emerging as a sustainable alternative for food production, with a biopsy of a live cow, theoretically one billion hamburgers could be obtained in 1.5 months, in contrast to the 0.5 million cows and 18 months needed in conventional methods. (Tomiyama et al., 2020).

Lab-grown meat production has the potential for land use reduction, as its facilities can be arranged vertically in laboratory environments, taking up less space compared to the extensive areas required by large farms (Tuomisto, 2019). The advantages of this is that the facilities are located in urban centres, which would help to minimise the costs associated with transport. In addition, it is estimated that greenhouse gas emissions from meat products could be reduced by up to 90%, accompanied by an 80% decrease in the use of other essential resources, such as land and water (Dantas & Pinto, 2023).

7. Advances in Cultured Meat Production

The first report on cultured meat was made public in 2008, and in 2013, several food critics tasted the first hamburger made by Dutch scientists in a laboratory, which had an approximate cost of manufacturing

$330,000. Israel-based pioneer in cultured meat production, Future Meat, has managed to reduce the price of a chicken fillet to $1.70, which is twice the cost of a conventionally produced one (Párraga, 2022).

The production of synthetic meat requires an essential culture medium called fetal bovine serum, which is obtained from calf stem cells. Annually, around half a million liters of this serum are produced, using up to two

million calf fetuses. The process involves slaughtering a mother cow, removing her uterus with the fetus, and obtaining stem cells using a needle inserted into the fetus's heart after slaughter (Moyano, 2021).

The implementation of technologies such as 3D printing, lab-based meat production, blockchain, vertical farming, as well as cell culture, has the potential to significantly alter current agricultural production. Some companies are already making strides in prototyping products such as chicken nuggets, sausages, and even foie gras. However, uncertainties remain about the proper naming and labeling to clearly distinguish them from products using real meat (Cruz, 2019).

For Cartín-Rojas & Ortiz (2018), lab-grown meat has the potential to meet the needs of highly nutritious protein sources, becoming a crucial ally in the fight against hunger and malnutrition that affect millions around the world. In the same vein, Chriki & Hocquette (2020) point out that, in order to meet the growing food demand projected for the year 2050, the FAO argues that it will be necessary to increase food production by 70%. In this scenario, it is proposed to grow muscle cells in a specific culture medium, highlighting so far a medium that includes FBS, which provides nutrients, hormones and growth factors in a highly effective way.

However, the current challenges facing man, such as climate change and the imperative need to feed an ever-increasing society, demand the exploration of alternatives in food production. According to Zuñiga et al., (2021), cellular agriculture emerges as a feasible initiative to address the growing demand for meat in a sustainable way, contributing to mitigate environmental problems, however, a significant challenge is posed that these products achieve levels of consistency and flavour comparable to those obtained directly from animals.

Likewise, Dantas & Pinto (2023) argues that cultured meat production brings notable benefits, such as reduced greenhouse gas emissions, reduced consumption of energy and natural resources such as water and land, as well as reduced use of antibiotics and pesticides. Similarly, Zuñiga et al., (2021), point out that meat generated through cellular agriculture would require less than one-tenth of the water and land compared to conventional production, resulting in lower greenhouse gas emissions and promoting a more responsible use of natural resources. As a result, cellular agriculture products have a lower environmental impact, are safer, and can be made in controlled, sterile, and safe environments.

Finally, for Cartín-Rojas & Ortiz (2018), the most significant limitation to the wide adoption of in vitro meat technology lies in its current cost of production, therefore, greater investment and more in-depth scientific research are required to make this technology more accessible on a large scale. On the other hand, Chriki & Hocquette (2020), argue that, despite the current high price, the production costs of cultured meat are likely to decrease in the near future, this decrease in costs could contribute to its acceptance by consumers. Therefore, cultured meat will face competition from other meat substitutes already on the market and better accepted by consumers, such as plant-based products.

8. CONCLUSION

Lab-grown meat production is a promising alternative to address the challenges associated with conventional meat production. The global demand for food is on the rise, and cultured meat offers the possibility of meeting this demand in a sustainable and ethical way, in addition, this technology makes it possible to reduce the environmental impact by decreasing the dependence on intensive animal farming, deforestation and greenhouse gas emissions associated with conventional livestock farming.

From a nutritional point of view, cultured meat is designed to replicate the sensory characteristics and nutritional value of traditional meat, different culture media, cell substrates and bioreactors are explored to ensure efficient muscle cell growth. This innovative product allows precise control of the nutritional profile, by adjusting the levels of proteins, fats and other nutrients to meet the specific needs of consumers, in addition, as it is produced in controlled environments, the risk of contamination and the presence of residues is reduced.

Cultured meat production is emerging as a sustainable solution that could contribute to food security and reduce the environmental impact of the food industry.

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