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REVIEW ARTICLES

# Is the development of low-cost media one of the greatest challenges to produce cultivated meat on an industrial scale?

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**Highlights**

- Traditional culture media for animal cells contain animal-derived ingredients
- Serum-based media are associated to ethical, safety, and economic concerns
- Alternative “animal-free” ingredients are key inputs in cultivated meat production
- Cost reduction of culture media is one of the greatest challenges for process scale-up

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**Abstract:** Cultivated meat (CM) has emerged as an “ethical” alternative for the consumption of meat, avoiding animal slaughter and safeguarding animal care. The idea behind animal cell cultivation, differentiation and proliferation is old, but the investments, technological developments and first efforts to produce CM on industrial scale are very recent. There are many challenges and bottlenecks within this new market, including social, environmental, technological, regulatory and logistic aspects; however, the emphasis of this article is the composition of the culture media for animal cells development, which is strongly attached to economy (component costs) and ethics (components of animal origin). Traditional basal media (such as Eagle´s Minimum Essential Medium and Ham´s F-12) comprise energy and carbon sources, vitamins, amino acids and trace elements; but the requirements for development and differentiation of skeletal muscle cells demand other components of animal origin, such as fetal bovine serum and/or other growth factors, hormones and inducers. Recent articles and patents have reported the substitution of these components, including the use of recombinant albumin, postbiotics, and microalgal extracts. Despite these efforts, the current market of CM is still in its “first childhood” with 107 enterprises around the world, and just a few of them are authorized to commercialize CM; the current price to the final consumer is, in the best case, 7.5 times higher when compared to traditional meat. Therefore, from our point of view, there is still a long way to go in developing this new product and establishing a new global market.

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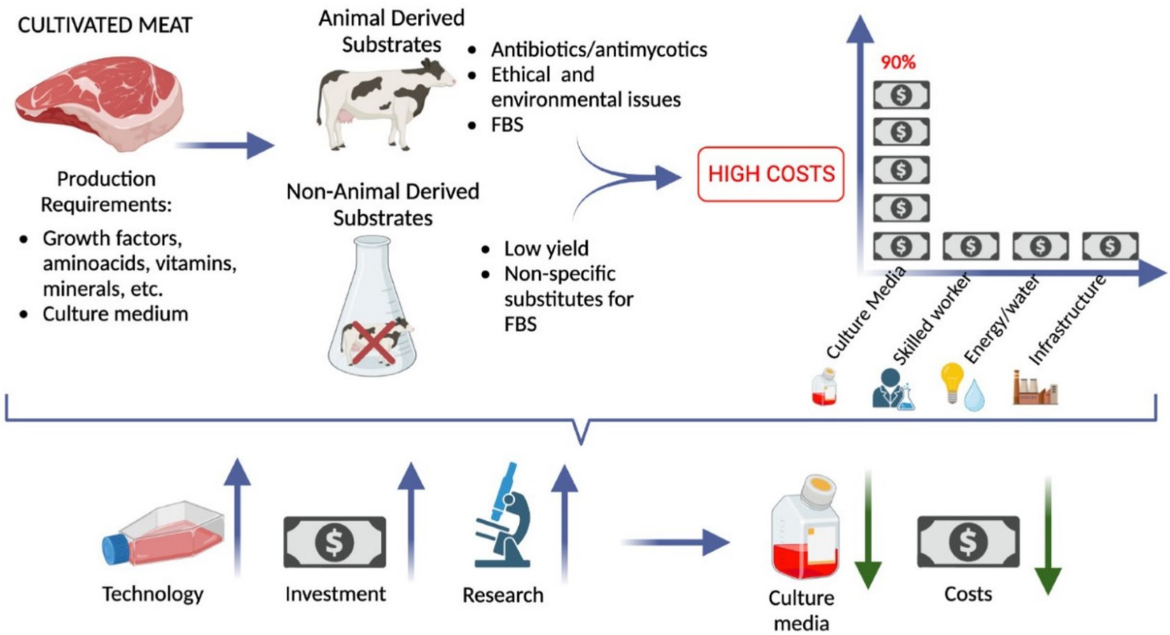
## Graphical Abstract




## Introduction

Cultivated meat (CM) from animal cell culture is a rising technology that is attracting the attention of investors all over the world. Only in 2021, US $ 1.38 billion was invested in the CM industry, representing roughly 71% of the total amount invested in history (Good Food Institute, 2021). This is given by a wide range of global challenges emerging from intensified population growth, climate change, and urbanization (Fernandes et al., 2022). It is expected that by 2050 the consumption of meat will increase greatly up to the point that the conventional meat supply will not be enough to meet the world's demands (Pandurangan & Kim, 2015).

Thereby, CM is an alternative to traditional methods that exploits the *in vitro* potential of stem cells to multiply and differentiate into specific tissues. The great advantages of CM compared to meat derived from animals are the ethical gains, once the slaughter of animals is avoided. Similarly, it presents itself as an alternative to the impact of conventional animal meat production on the environment and natural resources, leading to a considerable reduction in greenhouse gas emissions, water and land use (Letti et al., 2021; Post et al., 2020).

Despite the rising investments, the ethical issues, and the promising environmental advantages, various challenges have yet to be surpassed to scale up the process of CM production. One of the greatest concerns is the cost of culture media, as these are crucial for cellular functions, survival and proliferation (Yao & Asayama, 2017). Moreover, it has been reported that the cost of the culture medium may account for 55%-95% of the total marginal cost of the final product (Specht, 2020), which has a substantial effect on the economic feasibility of the process.

Before the conception of CM, cell culture technology took huge steps in several technical issues related to culture media. Advances were provided in a wide range of applications: on the study of basic cell biology, cellular mechanisms, functions and interactions; characterization and testing of compounds and their toxicity in cells (Verma et al., 2020); production of vaccines and biopharmaceuticals; cell engineering (Gadgil, 2017) and more. Table 1 presents the historical advances concerning the development of culture media and technologies for animal cells. The first medium for *in vitro* animal cell culture was proposed in the nineteenth century. It was designed to keep a frog's heart beating after dissection and removal, and its components were essentially a solution of salts that resembled the frog's body fluids (Ringer, 1882). After that, researchers began to focus on cells in culture devices and attempted to maintain the cells for longer periods (Yao & Asayama, 2017).

Nowadays, the complete formulation of a culture medium intended for animal cell culture comprises a complex set of components. Basically, the medium is composed of inorganic salts, nitrogen sources (amino acids), energy sources (glucose, fructose), vitamins, fat and fat-soluble components (fatty acids, cholesterols), nucleic acid precursors, growth factors and hormones, antibiotics, buffering systems, and specific oxygen and carbon dioxide concentrations (Verma et al., 2020).

Given its complex nature, it is reasonable to believe that the culture medium plays a key role in animal cells and tissue culturing once it must supply every essential nutrient to support cell growth and metabolic needs, to proliferate and further differentiate (Brunner et al., 2010). From the very beginning, almost all cultivation methods required animal serum (Lobo-Alfonso et al., 2010), *e.g.*, fetal bovine serum (FBS), which is considered to be a universal growth supplement for cell culture, being effective for most types of animal cells (Gstraunthaler et al., 2013). Despite the convenience of FBS, some drawbacks must be taken into

**Table 1.** Advances in culture media for animal cells and related technologies.

| Year | Advance | References |
|---|---|---|
| 1882 | Development of the first balanced salt solution to maintain animal cells | (Yao & Asayama, 2017; Ringer, 1882) |
| 1907 | First use of animal fluids (frog lymph) for animal cell growth | (Harrison et al., 1907) |
| 1908 | First culture of mammalian somatic cells | (Yao & Asayama, 2017) |
| 1910 | First use of blood plasma (chicken) for tissue cultivation | (Burrows, 1910; Carrel, 1912) |
| 1911 | The addition of amino acids and glucose was found to be better than using only balanced salt solutions | (Lewis & Lewis, 1912) |
| 1912 | Development of new culture flasks and discovery of the possibility of long-term cultivation with periodic exchange of media | (Carrel, 1912; Carrel, 1923) |
| 1913 | First successful use of tissue extracts (embryonic extract) for maintaining and growing of animal tissue *in vitro* | (Carrel, 1913) |
| 1922 | A bigger understanding of the importance of carbohydrate and protein fractions | (Lewis, 1922; Baker & Carrel, 1926) |
| 1933 | First report on the effectiveness of vitamins and hormones (insulin and thyroxine) for fibroblast culture | (Vogelaar & Erlichman, 1933) |
| 1951 | Development of an immortal cell line (HeLa Cells), which provided great advances in culture media design | (Yao & Asayama, 2017) |
| 1952 | First reports on the use of growth factors | (Levi-Montalcini, 1952) |
| 1955 | Acknowledgment of the quantitative need for amino acids and vitamins by HeLa cells | (Eagle, 1955) |
| 1959 | Development of the minimum essential medium (MEM) by Eagle, and further Dulbecco's modified MEM (DMEM) | (Eagle, 1959; Dulbecco & Freeman, 1959) |
| 1963 | Development of Ham's media, the first comprising not the whole serum, but proteins purified from it | (Ham, 1963) |
| 1965 | Development of Ham's F12 media, a completely synthetic medium with defined composition | (Ham, 1965) |
| 1976 | Findings about the need for hormones, selenite, albumin and transferrin, to develop a serum-free medium | (Hayashi & Sato, 1976; McKeehan et al., 1976; Guilbert & Iscove, 1976) |
| 1979 | Propositions to combine basal media, such as Ham's F12 and DMEM, for improved performance | (Barnes & Sato, 1979) |
| 1986 | Advances provided the possibility to commercially produce recombinant pharmaceuticals through animal cells | (Yao & Asayama, 2017) |
| 1995 | Development of a serum-free medium for large-scale cultures containing soy peptone | (Keen & Rapson, 1995) |
| 2000 | Research efforts to find low-cost and scalable alternatives for serum-free media | (Yao & Asayama, 2017) |
| 2009 | Development of a serum-free medium containing soy, wheat and yeast hydrolysates for recombinant protein production | (Kim & Lee, 2009) |
| 2012 | Development of cheaper substitutes for growth hormones | (Hasegawa et al., 2012) |
| 2016 | Creation of the Good Food Institute (GFI), a non-profit organization that aims at the development of a sustainable, secure, and just protein supply, including cultured meat | (Good Food Institute, 2021) |
| 2019 | Stakeholders' collaboration to boost the cultured meat industry, e.g., the Cultivated Meat Modeling Consortium (CMMC) and other innovation groups | (Kahan et al., 2020; Merck Group, 2022) |

**Source:** Adapted from Yao & Asayama, 2017.

consideration. The main ethical concern with the use of FBS is the method of harvesting and processing, which requires the puncture in the heart of a cattle fetus that had previously been separated from its progenitor (Brunner et al., 2010; Gstraunthaler et al., 2013). Furthermore, it is essential to emphasize some technical disadvantages: the non-specific qualitative and quantitative composition of the serum (Baker, 2016), the potential presence of prions (Chou et al., 2015), endotoxins (Pilgrim et al., 2022), microorganisms, cell growth inhibitors (Brunner et al., 2010; Gstraunthaler et al., 2013), and the unpredictable reduction in the market offer of FBS, resulting in an increase of market value (Subbiahanadar Chelladurai et al., 2021).

Transitioning to serum-free media is essential to the advance of the CM industry and the use of animal-derived components must be reduced to ensure the quality and safety of the products. However, the costs associated with this transition must be evaluated. Replacing growth factors, animal-derived proteins and including a buffer system represent significant economic challenges of serum-free media once the supply and availability of recombinant components are limited (Specht, 2020). Furthermore, the need to replace the medium after 2–6 days due to the accumulation of toxic metabolites is an additional cost. Another contribution to the escalating prices is the need for research and development of new products and technologies, as well as the requirement of a highly qualified and multidisciplinary team composed by professionals of various areas, such as engineering, biotechnology, logistics, product development and more (Garrison et al., 2022). As a pivotal component of the CM production process, culture media development must focus on cost reduction and consistency of the inputs, factors that are critical to scale up the process to facilities that can meet the market demand. As a result, the search for culture media that are low-cost, scalable, food-safe, and animal-free, is critical (Stout et al., 2022), and has led to the emergence of a range of alternative cell-culture media.

This review addresses some aspects, challenges and advances in CM production, focusing on the development of culture media. Traditional substrates for animal cell culture are described, and alternative medium compositions free of components of animal origin are reviewed, based on recent publications and patents on CM production.

## Commercial production of cultivated meat – current challenges and regulation

Despite being considered a possible solution to the problems faced by agriculture, CM still confronts numerous challenges that make its scalability and cost reduction unfeasible (Good Food Institute, 2021; Guan et al., 2022). The culture medium is linked to several of these challenges mainly due to the nutritional requirements of animal cells. Traditional media usually consist of a variety of animal sera such as horse serum or FBS, in addition to many other components that are necessary to induce cell proliferation, such as growth factors, vitamins, salts, amino acids and others. However, the use of serum is associated with experimental irreproducibility, increased bacterial and viral contamination in the process, risk of contamination with prions, increased costs and still hinders the original intention of avoiding animal breeding and slaughter in the production of CM (Stout et al., 2022; Good Food Institute, 2022b; Ye et al., 2022). Furthermore, the use of antibiotics and antifungals in the composition of traditional media can alter gene expression (Ryu et al., 2017), proliferation (Kolkmann et al., 2020), and differentiation of animal cells (Chang et al., 2006), besides promoting antimicrobial resistance in their consumers (Good Food Institute, 2022a). Therefore, the great challenge today is to develop alternative culture media that do not require the use of animal-derived factors and antimicrobial compounds, but are still capable of providing the same cellular responses obtained with traditional media. Alternative “animal-free” media for CM production will usually demand recombinant proteins and other costly compounds, in this sense, the development of lower-cost compositions is determinant for process viability (Stout et al., 2022).

Besides the composition and cost of culture media, other challenges associated to CM production are related to scalability and process costs, the need to establish a reliable supply chain for the materials and equipment used, the creation of regulations that allow the marketing of CM, ethical and environmental impasses, and consumer reluctance to the lab-cultivated product (Stout et al., 2022; Good Food Institute, 2022c; Singh et al., 2022).

In almost all areas where an emerging technology intersects with a heavily regulated industry, there will always be barriers to be overcome in the face of existing regulatory burdens, and CM is no different. In this sense, CM will test the trust of the current regulatory system in some regions, such as in the United States (US), where CM would not be considered a “meat product” according to the Federal Meat Inspection Act (FMIA). The FMIA assumes that a meat product is derived from a carcass, and if the FMIA is not applicable, another regulatory scheme is necessary to ensure the safety of CM for consumption, as well as the process of product checking and labeling (Sanchez, 2018). With this dilemma, the US Food and Drug Administration (FDA) and the United States Department of Agriculture (USDA) come in to tackle regulatory issues. On November 16, 2018, the USDA and FDA announced their intention to implement a joint regulatory framework for CM. Less than a year later, on March 7, 2019, the FDA and USDA released a formal agreement describing their respective roles and supervision responsibilities under this framework and how they will collaborate to regulate the production of CM and its entry into commerce (Good Food Institute, 2021).

On November 17, 2022, the FDA reported, for the first time, a lab-developed meat product from the California-based startup Upside Foods as safe for human consumption, marking an important milestone for CM eventually becoming available in US supermarkets and restaurants. The FDA allowed the use of animal cell culture technology to take living cells from chickens and grow them in a controlled environment, with the aim of producing cultured animal cell food. The agency said it had evaluated Upside Food’s cultured cell production and material, and had “no further questions” about the safety of their cultivated chicken fillet. The company will be able to bring their products to market once they are inspected by the USDA (CNBC, 2022). Facing the United States, taking the lead on regulatory issues of cultivated meat, is Singapore. On December 1$^{st}$, 2020, the Singapore Food Agency (SFA) approved the sale of cultivated chicken from Eat Just Inc. – the first approval of its kind in the world. The SFA is currently assessing other local CM and seafood startups, including Shiok Meats and Ants Innovate, on potential approval of their products, and is open to working with the companies from the early stages of research and development of the products (Good Food Institute, 2021). Aligned with the same goal, other countries are also following the path of CM regulation, such as Canada, Australia, and New Zealand, which share the same regulatory body – the Food Standards Australia New Zealand (FSANZ) – in addition to the European Union, the United Kingdom, Japan, Israel, China, and Brazil.

In Brazil's case, the regulatory agency is the National Health Surveillance Agency (ANVISA), which is committed to understanding the challenges of food safety and labeling of CM and is in the process of developing a regulatory framework that includes CM products. According to ANVISA, Brazil intends to adopt a similar model to that of the US and the European Union. Companies will first include information about their product from the beginning of the research and development process. Afterwards, ANVISA will assess the product's safety according to the regulatory framework for new foods (Good Food Institute, 2021). As stated previously, the use of antibiotics in culture media for CM production is one of the many challenges encountered. Most CM regulations are under development or constantly being updated around the world, so no legislation was found to set limits on the use of antibiotics in culture media for food production in Brazil. However, there are regulations that approach this purpose, with ANVISA Instruction Nr. 51 being the one that best fits.

ANVISA Instruction Nr. 51, dated December 19, 2019, establishes the list of maximum residue limits (MRL), acceptable daily intake (ADA) and acute reference dose (ARD) for active pharmaceutical ingredients (API) of veterinary medicines in animal-derived foods (Agência Nacional de Vigilância Sanitária, 2019). Through this regulation, the MRL are defined as the maximum concentrations of veterinary medicine residues in animal-derived foods. The accumulation of drug residues in animals occurs when they are treated with drugs throughout their growth, due to diseases or for disease prevention, and for this reason it is necessary to control the existing amount of these drug residues in the final product, once they can be harmful to humans (Agência Nacional de Vigilância Sanitária, 2023). The standard brings 240 active pharmaceutical inputs, of which MRLs need to be established, with most of these limits being established through existing foreign regulations regarding these substances and their respective maximum allowed amounts for consumption, sourced, e.g., from the European Medicines Agency (EMA), the US FDA, Health Canada, the Australian Pesticides and Veterinary Medicines Authority (APVMA), and the Japan Food Safety Commission (FSC) (Agência Nacional de Vigilância Sanitária, 2021).

## Traditional substrates and media components for cultivated meat production

For animal cell cultivation, the use of commercial basal media is a common practice that ensures reproducibility and solid scientific control. These media formulations are applied in several studies with different cell types and culture protocols (Bain et al., 2013; Ho et al., 2014; Cui et al., 2018; Park et al., 2021; Li et al., 2021; Joo et al., 2022). The gold standard types are Eagle's Minimum Essential Medium (MEM) (Eagle, 1959), Dulbecco's Modified Eagle's Medium (DMEM) (Dulbecco & Freeman, 1959) and Ham's F-12 (Ham, 1965). They provide in their composition most of the basic components needed for cell survival and growth, including energy source (glucose, L-glutamine), amino acids, vitamins, ions, and trace elements (sodium, calcium, magnesium, potassium, iron, manganese, zinc, selenium, and copper) (Price, 2017; O'Neill et al., 2021).

The basal medium, however, requires supplementation with animal-derived factors to provide adequate mitogens for cell proliferation (O'Neill et al., 2021). According to Merck's own website, a supplier of culture media, it is recommended that, for the growth of primary skeletal muscle cells, the basal medium is supplemented with calf serum 0.05 mL/mL, fetuin (bovine) 50 µg/mL, epidermal growth factor (human recombinant) 10 ng/mL, basic fibroblast growth factor (human recombinant) 1 ng/mL, insulin (human recombinant) 10 µg/mL, and dexamethasone 0.4 µg/mL for the cell growth or proliferation medium; for the differentiation medium only insulin (human recombinant) 10 µg/mL is suggested. Despite the possibility of obtaining some components through recombinant technologies, there is a marked presence of components of animal origin that are difficult to replace, such as FBS (Price, 2017; O'Neill et al., 2021; Singh et al., 2022).

Historically, the serum used in cell culture has bovine origin, and may be of fetal origin, of newborn calf (less than 3 weeks of life), calf (between 3 weeks and 12 months of life) and adult (over 12 months). The most common to be used in cell culture is FBS which has advantages such as high levels of substances that promote cell growth, low level of immunoglobulins in relation to oxen serum in other stages of growth and low amounts of complements (Nims & Harbell , 2017; Warner, 2019). This component provides a variety of factors for cell growth, such as: hormones, accessory and binding factors, membrane permeability regulators, lipid enzymes, micronutrients, trace elements, buffers, free radical scavengers, neutralizers of enzymes and toxins, mitogenic growth factors (Price, 2017). Despite the immense variety of compounds important for cell growth, serum can present risks such as the presence of microbial contaminants (i.e., bacteria, fungi, mycoplasmae, or viruses) or prionic contaminants. Another associated risk is the variation from one batch to another, because it is a complex source that can add numerous performance variables (Nims & Harbell , 2017). In addition, its high cost, reaching more than US $ 2000 for the production of 1 kg of tissue, makes it impossible to use in CM production (O'Neill et al., 2021; Stout et al., 2023). In some cases, it is also used as a supplement to bovine serum albumin (BSA), which similarly provides the medium with growth factors, hormones, amino acids, and proteins that are beneficial to embryonic development (Zuelke & Brackett, 1990).

Horse serum is also a compound of animal origin widely used in the differentiation culture of bovine cells (Torgan et al., 2000; Liu et al., 2019). This serum contains the necessary factors to induce differentiation of myoblasts such as hormones, accessory and binding factors, membrane permeability regulators, lipids, enzymes, micronutrients, trace elements, buffers, free radical scavengers, neutralizers of enzymes and toxins, and mitogenic growth factors (Price, 2017). Its use in chicken cell cultures has been reported

(Joo et al., 2022), being added to the basal composition together with FBS. Similar to FBS, horse serum holds ethical problems in obtaining it as well as technical problems of lot variation, being one of the components to be replaced in the formulation of more appropriate media for industrial processes. Another serum type, used for fish cell culture, is the trout serum (Ho et al., 2014). In the same way as the others, it provides the necessary components for cell development, such as growth factors, proteins, regulators, micronutrients, among others (Blaxhall, 1985; Manera, 2021). However, several studies demonstrate the variability of the chemical composition of this serum (Amend & Fender, 1976; Olesen & Jørgensen, 1985; Manera, 2021).

It is also worth mentioning the presence of antibiotics or antimycotics in several media reported in CM production studies (Hong et al., 1996; Bain et al., 2013; Ho et al., 2014; Hanga et al., 2020; Li et al., 2021; Joo et al., 2022; Park et al., 2021; Stout et al., 2022), used in order to avoid contamination in the culture medium. For academic purposes, on a small scale, the use of antibiotics is commonplace, but for applications in the food industry this is not recommended, since the "World Health Organization strongly recommends a global reduction in the use of all classes of antibiotics of medical importance in food-producing animals". This is due to the increase in microbial resistance phenomena and the sensitivity of some consumers to specific antibiotics, generating an obstacle even to the acceptance of the product by the final consumer (Kuhlmann, 1995; Relier et al., 2016; O'Neill et al., 2021).

## Alternative sources for sustainable processes of cultivated meat production – "animal-free" media

Alternative substrates of non-animal origin are essential inputs for the development of sustainable processes of CM production. The use of serum-free media, besides avoiding the sacrifice of animals, promises to reduce the high costs of serum-based media, the variation of the product among lots and the risk of the presence of viruses and prions, which improves the safety of CM consumption (Kadim et al., 2015; Allan et al., 2019; Zhang et al., 2020).

The serum-free medium typically consists of a basal medium supplemented with a combination of essential factors and inducers for cell growth and differentiation, preferably of non-animal origin. In the laboratory, the most popular substitutes for FBS are sericin protein and platelet lysates (Guiotto et al., 2020; Chelladurai et al., 2021). However, these are also derived from animals and ideally should not be used for CM. The solution in this case is also the biggest challenge: the development of a chemically defined medium that yields the same cellular response of serum-rich media. There is no consensus in the literature on the best approach to achieve this result, since FBS is a complex supplement composed of a set of several macromolecules and there is no single ingredient that can completely replace it (Chelladurai et al., 2021; O'Neill et al., 2022). One should also keep in mind that depending on which supplement is added to the formulation of the medium and changing its concentration, it is possible to select cell types and induce specific cellular behaviors, such as differentiation and proliferation (O'Neill et al., 2022). For example, in relation to the cultivation of bovine satellite cells, one of the most promising cell types for cultivated beef (Warner, 2019; Guan et al., 2022), Stout et al. (2023) described a method to specifically expand these cells by adding recombinant albumin to a pre-existing serum-free medium (Kuo et al., 2020) designed to induce the growth of pluripotent stem cells. As albumin is the most abundant protein in serum, its substitution, especially by a type of recombinant origin, can lead to results comparable to those of serum-based media (Yao & Asayama, 2017). Subsequently, an update of the medium was further elaborated, altering it to replace recombinant albumin with rapeseed protein isolated from isoelectric protein precipitation (Stout et al., 2023). In addition, the substitution of serum by the so-called plant sera, which are protein fractions of plant extracts (Pazos et al., 2004), has been studied. In this sense, there are also reports on microalgal extracts, such as the use of *Chlorella vulgaris* extract to replace most basal nutrients for animal cells, including glucose, amino acids, and vitamins. The authors were able to demonstrate that the proliferation rate after six days of culture and differentiation of primary bovine myoblasts were comparable to the commonly used basal medium DMEM (Okamoto et al., 2022).

Defined and commercial serum-free media are available. However, as presented by the analysis of Kolkmann et al. (2020), when compared with the basal growth medium for proliferation, they have a deficit in cell growth. In summary, only three commercial media (FBM, FBM/DMEM and Essential8TM) presented a potential to become as effective as the serum-based medium for proliferation. Nonetheless, improvements are still needed to increase survival, density and cell fixation (Kolkmann et al., 2020). Other than that, the costs of such media are of great concern and, depending on which commercial basal media is used, the price is in the range of US $ 340–680 per liter.

The possibility of developing a chemically defined medium, such as those commercially available, would be an alternative, as presented by Kolkmann et al. (2022). In their study, a serum-free medium with animal components was defined for the cultivation of bovine primary satellite cells. The composition consisted of basal medium DMEM/F12, supplemented with ascorbic L acid 2-phosphate, fibronectin, hydrocortisone, GlutaMAXTM, albumin, insulin-transferrin-selenium-ethanolamine (ITS-X), interleucine-6 (hIL-6), alpha-linolenic acid and growth factors such as fibroblast-like (FGF-2), transforming growth factor beta (TGF-β), vascular endothelial (VEGF), insulin-like (IGF-1), hepatocyte (HGF) and Palet-derived (PDGF-BB). According to Reiss, Robertson and Suzuki (2021), the use of growth factors as essential supplements to chemically defined media is responsible for 90% of total medium costs. For example, the use of FGF-2 and TGF-β accounts for US $ 150–200, for each growth factor, per liter of standard medium concentration. In addition, hydrolyzed products are used as substitutes for FBS (Ho et al., 2021). The use of cyanobacteria hydrolysates (Tuomisto & Teixeira, 2011) has been reported to support muscle cell growth, although there is concern about safety due to the potential cytotoxicity. Another study, also targeting bovine muscle cells, reported the use of pork plasma digested with alcalase for its growth. However, the animal element still remains, and its use for CM is not recommended. Therefore, plant hydrolysates and yeasts are used, as already used for CHO cell cultivation (Hu et al., 2018; Zhang et al., 2019; Ng et al., 2020; Montserrat-de la Paz et al., 2020), an option that is potentially safer and more economical (Lobo-Alfonso et al., 2010; Ho et al., 2021).

For avian meat specifically, the patent filed by the company Eat Just Inc. (US20200392461-A1, 2020) describes a medium with low serum concentration (less than 2%) for chicken fibroblast culture containing fatty acids and growth factors. The cells were first conditioned to thrive under low concentrations of FBS and right before removing the serum completely, fatty acids and IGF, EGF, FGF were added to the medium. Cell growth and viability obtained by this method were similar to a culture containing ideal FBS concentration.

As for the porcine cell culture, more studies are needed for the development of alternatives to the use of serum. Although most patents and articles still report the use of this component, there are scientists and companies engaged in reducing the use of serum during pork cell cultivation. Štěpánová et al. (2022) were able to culture porcine monocyte-derived macrophages using Nu-Serum™ growth medium supplement (NUS), which is a low-serum alternative. The Dutch start-up company Meatable (2022) produced cultivated meat without the use of FBS in 2018 and is expected to sell pork sausages in 2025. The patent filed by Memphis Meats, Upside Foods, the University of Missouri and the PETA organization (WO2015066377-A1, 2015) described the use of N-2 and B-27 supplements as a serum replacement for self-renewal and terminal differentiation of a myogenic-transcription-factor-modified porcine cell line (Genovese et al., 2021).

Compared to the culture of terrestrial vertebrate animal cells, the culture of aquatic animal cells still needs further study, especially for process optimization and large-scale production. For this purpose, fish embryonic stem (ES)-like cells, a term that encompasses cells with the least differentiation capacity and the embryonic stem cells (ESCs) themselves, are usually used (Good Food Institute, 2022b).

The common culture medium for fish (ES)-like cells is ESM1, composed of FBS, fish serum, fish embryo extract, and human growth factors (FGF2 and LIF) (Hong et al., 1996). However, aiming to eliminate animal-derived components in the culture, the components of serum and embryo extracts have been studied separately in order to analyze their importance for ES cell growth and to be added in serum-free media formulations (Good Food institute, 2022b).

So far, there are few reports of serum-free media formulations for fish cell culture that can maintain adequate proliferation rates. One approach described the complete adaptation of channel catfish ovary cells (CCO) from a serum-containing medium to a serum-free medium by gradually reducing the concentration of this compound in the medium. As a result, the cells maintained their growth, morphology, and nutritional characteristics unchanged (Radošević et al., 2016). Further studies are necessary to be able to culture fish cells in serum-free media and to broaden these formulations to other commercially known species effectively.

## Patent search on serum-free media for cultivated meat production

A patent search was carried out in the Derwent Innovations Index Database, on December 13th, 2022, using a combination synonyms of "cultivated meat", namely [artificial or synthetic or *in vitro* or *ex vivo* or cell based or (cell)-culture(d) or vat grown or lab(oratory)-grown or animal free or slaughter free or animal cell or test tube or clean or engineered or cellular or *in vitro* cell(ular) cult(ure)] and [meat or beef or steak or flesh or shmeat or frankenmeat], and their orthographic variants, together with terms associated to culture media [serum-free or FBS-free or culture (cultivation) medium (media) or substrate], and their orthographic variants, in the field Topic, without limiting a time frame. This search resulted in 133 patent documents, of which 17 were in fact related do media compositions. These will be described as follows.

The patent document WO2021148955-A1, filed in 2021 by the Yissum Research and Development Company of the Hebrew University of Jerusalem and Future Meat Technologies (Israel), describes a cell culture medium supplement devoid of animal protein and component, used for producing cultured meat. The supplement comprises plant protein homologue of a serum protein. The so-called plant albumin is derived from the water-soluble fraction of a plant protein isolate, bearing a molecular weight of 13–110 kDa and is used in the culture medium at the concentration of 0.01–10 wt.%. The composition also comprises a plant catalase (50–70 kDa, 100 ng/mL in cell culture medium), a plant fibronectin (40–60 kDa, 0.1–100 pg/mL), and a plant insulin (0.05–10 pg/mL). The plant albumin is selected from chickpea, hemp seed, lentil, pea, soy, wheat or potato, preferably pea albumin or a potato albumin. The plant catalase is obtained from *Arabidopsis*, cabbage, cucumber, cotton, potato, pumpkin, spinach, sunflower, tobacco or tomato. The plant fibronectin is derived from bean, chickpea, lentil, rice, soy, tobacco or wheat. The plant insulin is glucokinin, charantin, or corosolic acid. The patent document WO2021148960-A1, filed by the same assignees in 2021, describes a serum-free medium composed of carbohydrates, amino acids, vitamins, minerals and serum replacement components including albumin, growth factors, enzymes, attachment factors and hormones.

Plant extracts are often applied to replace ingredients of animal origin in media for CM production. The patent WO2022211461-A1 (2022, HyupSung University and Danagreen, South Korea) reports the use of curcumin (0.01–100 μM, preferably 8 μM), glycine (0.1–1000 mM, preferably 100 mM) or insulin (0.01–100 μM, preferably 10 μM) in a medium free of basic fibroblast growth factor to cultivate muscle stem cells. The patent documents KR2022135627-A and KR2022135628-A (2022, Korea Food Research Institute, South Korea) propose the use of Lycii fructus (gogi berry) extract and of Mori fructus and Xanthii fructus extracts, respectively, to reduce the use of FBS in the cultivation of muscle satellite cells. The patent EP4043551-A1 (2022, Buehler, Switzerland) describes a composition for cultured meat production containing an acid hydrolysate of plant material (rice bran, wheat bran, rye bran, maize bran, spelt bran, or brewer's spent grain) and a food grade premix comprising one or more minerals, vitamins, amino acids, and/or salts. There is also a patent document (KR2021090560-A, 2021, Sejong University, South

Korea) proposing the use of milk serum as an alternative to FBS. However, this component is of animal origin and was effective in partially replacing FBS, exhibiting cell growth ability that resembles of 10% FBS at a level of 7.5% treated alone, thus reducing the cost of cultured meat production. The composition was prepared by mixing milk powder with water, treating with UV light, centrifuging, ultrasonically processing the intermediate layer, treating with UV rays, and filtering the obtained product. In the patent document WO2021151025-A1 filed in 2021 by Air Protein and Cipo (USA), the serum-free culture medium is prepared from microbial protein hydrolysates, where the microorganism is chemoautothrophic and genetically modified, is able to use $CO_2$, CO, and $CH_4$ as carbon sources, and is selected from *Aquifex* sp., *Cupriavidus* sp., *Corynebacterium* sp., *Gordonia* sp., *Nocardia* sp., among several other species. The patent US2022098546-A1 (2022, Biftek, USA) reported the use of postbiotics as supplements to be added to a culture medium for stimulation of cell growth and proliferation, as cost-effective substitutes of FBS. The postbiotics are derived from strains of lactic acid bacteria, bifidobacteria, spore-forming bacilli, and yeasts, and the supplement further comprises bovine serum albumin and sericin. Other patents reporting microbial sources of nutrients are CN114703126-A (2022, Shanghai CellX Biotechnology, China), that describes the use of hydrolysed bread yeast together with fibroblast growth factor 2 for the cultivation of muscle stem cells; CN115125195-A (2022, China Ocean University, China), that reports the preparation of single cell green algae combination functional factor (namely an aqueous extract of *Chlorella*) for replacing serum in cell culture meat, particularly fish muscle cells; and KR2022040417-A (2022, Yonsei University, South Korea), that describes a culture solution composition comprising egg white, sericin, and microalgae-derived ingredients, where the microalgal active ingredient is C-phycocyanin, for the production of cultured meat, particularly through the cultivation of myoblasts isolated from livestock, myocyte, fibroblast, adipocyte, myosatellite cell, mesenchymal stem cells, induced pluripotent stem cells, satellite cells, adipose-derived stem cell, or embryonic stem cells. The patent document CN112210525-A (2021, Jiangnan University, China) describes the detailed composition of a serum-free medium for the cultivation of animal stem cells (including embryonic stem cells, muscle-forming stem cells, mesenchymal stem cells or adipose-forming stem cells), animal muscle cells or animal fat cells. The medium contains preferably 130 mM sodium chloride, 3 mM potassium chloride, 5 mM D-glucose, 12 mM sodium bicarbonate, 0.5 mM monosodium phosphate, 1 mM magnesium chloride, 2 mM calcium chloride, 0.5 mM succinimide hydrochloride, 130 mM cell growth factor, 3 mM phospholipid growth factor, 0.1 g/L transferrin, 0.5 mg/L lipid, 5 g/L amino acid, 5 mM biotin, 5 mM vitamin B12, 10 mM vitamin B6, and 0.2 mM vitamin C.

Some patent documents reported the use of recombinant molecules as ingredients in cultured meat production, as represented by WO2021245711-A1 (2021, ORF Liftaekni Ehf, Iceland), that proposed the use of a growth factor composition comprising at least one recombinant animal growth factor (keratinocyte growth factor, vascular epithelial growth factor, fibroblast growth factors, among others) and at least one plant seed protein, where the growth factor is produced in a transgenic plant including barley, wheat, oat, rye, maize, rice, soya, peas, millets, sorghum and rape, and the seed protein includes dehydrins, protease inhibitors, hordeins, globulins, albumins, prolamins, violins, glutelins, zeins, among others; CN114874929-A (2022, Jiangnan University, China), describing the synthesis of heme in recombinant *Pichia pastoris*; CN113136349-A (2021, Jiangnan University, China), reporting the expression of heterologous hemoglobin and myoglobin in *P. pastoris*; and CN113549561-A (2021, Jiangnan University, China), showing the expression of heterologous hemoglobin and myoglobin in *Saccharomyces cerevisiae*. Table 2 summarizes the patent documents related to serum-free media for CM production.

## Market perspectives and cost estimates

Within this innovative universe of cultivated meats, whether chicken, fish, pork or beef, we clearly have a market on the rise. Every day it becomes clearer the accelerated growth of companies entering this area, acting from the development and advances in the final product to consumables and equipment aimed at the industry, such as cell banks, culture media, microcarriers/matrix and bioreactors. Starting in 2013, when the world's first cultivated meat burger was developed by Professor Mark Post (Maastricht University, Netherlands), which cost over US $ 280,000 at the time (CNBC, 2020; Good Food Institute, 2021), technological development has been rising exponentially.

There is an increasing number of companies over the years producing or about to start producing CM, as represented in Figure 1. In 2021 there were already more than 100 companies focused on this new segment. Most are located in the United States, covering 26 of the 107 companies worldwide. Israel, the United Kingdom and Singapore follow with 14, 12 and 9 companies, respectively. Despite the launch of several new companies around the world, few of them already have permission to commercialize CM, one of them being located in Singapore. At the end of 2020, the country obtained the approval of SFA for the sale of a chicken cultivated meat product by Eat Just (Good Food Institute, 2021; Good Food Institute, 2022d).

Compared to the aforementioned countries, Brazil is still developing CM technologies in early stages. However, it is an extremely promising country considering the high levels of production and consumption of meat. Ambi Real Food is the first Brazilian CM startup, located in Porto Alegre. There have also been significant investments from JBS, the world's largest protein producer. The company invested US $ 100 million in the purchase of Biotech Foods (a Spanish company) and in the creation of a research and development department focused on CM in Brazil. The idea is that the technology of the acquired startup will be shared with Brazil to accelerate the development of the Brazilian CM market (Good Food Institute, 2021).

The year 2021 registered important milestones in the CM universe. The Israeli-based Future Meat Technologies built a pilot plant with the capacity to produce 500 kg a day of cultivated chicken and pork meat through investments of around US $ 400 million. Another market giant, Upside

**Table 2.** Patent documents related to serum-free media for cultivated meat production according to technology type.

| Patent number | Assignee | Year | Country |
|---|---|---|---|
| | **Plant proteins** | | |
| WO2021148955-A1 | Yissum Research and Development Company of the Hebrew University of Jerusalem and Future Meat Technologies | 2021 | Israel |
| WO2021148960-A1 | Yissum Research and Development Company of the Hebrew University of Jerusalem and Future Meat Technologies | 2021 | Israel |
| | **Plant extracts** | | |
| WO2022211461-A1 | HyupSung University and Danagreen | 2022 | South Korea |
| KR2022135627-A | Korea Food Research Institute | 2022 | South Korea |
| KR2022135628-A | Korea Food Research Institute | 2022 | South Korea |
| EP4043551-A1 | Buehler | 2022 | Switzerland |
| | **Milk serum** | | |
| KR2021090560-A | Sejong University | 2021 | South Korea |
| | **Microbial extracts** | | |
| WO2021151025-A1 | Air Protein and Cipo | 2021 | USA |
| US2022098546-A1 | Biftek | 2022 | USA |
| CN114703126-A | Shanghai CellX Biotechnology | 2022 | China |
| CN115125195-A | China Ocean University | 2022 | China |
| KR2022040417-A | Yonsei University | 2022 | South Korea |
| | **Serum-free medium composition** | | |
| CN112210525-A | Jiangnan University | 2021 | China |
| | **Recombinant molecules** | | |
| WO2021245711-A1 | ORF Liftaekni Ehf | 2021 | Iceland |
| CN114874929-A | Jiangnan University | 2022 | China |
| CN113136349-A | Jiangnan University | 2021 | China |
| CN113549561-A | Jiangnan University | 2021 | China |


**Figure 1.** Main companies of cultivated meat around the world. **Source:** The authors, 2022.

Foods, opened in November 2021 a plant of 53,000 $m^2$ with a current production of around 23,000 kg/year, and an estimated increase in capacity to 180,000 kg/year in the future. The American Wildtype developed a pilot plant capable of producing approximately 90,000 kg/year. Based on the forecast made by Blue Horizon Corp., it is estimated that by 2030 the CM market will reach US $ 140 billion (CNBC, 2020; CISION, 2021; Good Food Institute, 2021).

When talking about prices for such products, we notice a huge difference when comparing the prices found for traditional meat and CM. In an article published in CNBC (2020), Jade Scipioni brings data provided by Bloomberg indicating that the production cost for a kilogram of CM ranges from US $ 400 to an incredible US $ 2,000.

In the mid-2021, Future Meat Technologies, a producer of cultured chicken meat and currently the only company commercially offering CM to the public, produced chicken breast without the use of animal sera at prices around US $ 16.98 per kilogram, according to Natália Berkhout in her report published in January 2022 in the digital magazine Poultry World (2022). Conducting research with the aim of comparing this information to the price of traditional chicken meat in the same period of the year, a report made by IndexBox showed that the price of chicken meat in 2021 reached the average of US $ 2.26 per kilogram (IndexBox, 2022). Using this average value, the cost of the CM is approximately 7.5 times higher than that of the traditional meat, exposing the huge discrepancy existing in this market. The trend is that each year, with novel technologies concerning culture media, bioreactors, microcarriers, scaffolds, and post-processing, the price to produce CM will reduce gradually. A hypothetical economic analysis carried out by the GFI and CE Delft presented an estimate that, by 2025, various regions will already have their regulatory issues regarding CM approval and the costs will be 10 to 100 times lower, reaching a parity of prices with that of conventional meat by 2030 (Good Food Institute, 2021).

## Conclusions and perspectives

As expected for new technologies, the CM industry has been established in a scenario of many promises, but with many uncertainties and challenges. Considering the economic aspect, it is imperial to reduce production costs. From industrial point of view, bioreactor design and process scalability are important concerns. From a logistical point of view, the large-scale supply of raw materials is a crucial factor. The technical challenges are countless, including the cultivation, proliferation and adhesion of animal cells, and the selection of scaffolds. Regarding consumer market, food safety and consumer acceptance are issues. Regarding legislation, preliminary regulation already exists in some countries, but they are quite recent and still need to be consolidated. However, the highlight of this article is related to sustainability and to the logic that guides the reduction/elimination of the slaughter and/or exploitation of animals: it deals with the composition of the culture media used for animal cell cultivation; in particular, the replacement of components of animal origin by alternative sources, derived from plants or microorganisms. We have shown that most culture media still depend on elements such as fetal bovine serum, growth factors, hormones, egg albumin, among others. There are also many research works and patents that propose the reduction of the amounts or concentrations of these components. Only a few consider the complete replacement of some of the components from animal sources by alternative sources (such as transgenic albumin excreted by rapeseed, postbiotics secreted by microorganisms, and extracts from microalgae). Although there are already some proposals with alternative sources, it is necessary to consider the costs of the components, the large-scale supply of some of the raw materials (mainly those obtained from transgenic organisms, as is the case of rapeseed albumin), and, evidently, of both regional and global regulatory and logistic aspects. Given this scenario, we conclude that the maturity of the CM industry still depends on numerous factors of different natures, but that certainly one of the most relevant is the definition of the components of the culture medium for animal cells, with emphasis on the need for cost reduction and for the supply of ingredients of non-animal origin. Therefore, we expect this work can help to guide and put some light for future research and technological developments in this field.

## Conflict of interests

The authors declare that there are no conflicts of interest.

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