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Biotechnological Applications to Produce Animal-free Meat and Milk: Ethical Considerations

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Introduction

Biotechnologies have consistently improved the production of many types of foods [1], including cheeses [2], wine [3], fruits [4], and crops [5]. However, new stem cell and gene editing biotechnologies may cause a paradigm shift in these industries. In this article, I explore the potential scientific, health, cultural/religious, ethical, and economic ramifications of using these innovative biotechnologies to make milk, meat and poultry products from non-traditional food sources.

Milk

Cow's milk is considered a staple in many people's diets. It is consumed as a beverage, poured on cereal and added to smoothies, tea or coffee. Cow's milk boasts an impressive nutrient profile. It's rich in high-quality protein and important vitamins and minerals, including calcium, phosphorus and B vitamins. The history of including milk in the human diet is rich and fascinating [6]. Milk consumption has been part of the human diet for thousands of years. In ancient Egypt milk and other dairy products were generally reserved for Royalty or the wealthy. There are many reasons and theories why milk became such a popular food in western countries. Some believe that as infant mortality rose in American cities in the 1800's milk emerged as a healthy food for children. Clearly US government sponsored ads over the past 40 years has influenced the acceptance of milk as a staple of the western diet.

However, there are many health-related concerns associated with the consumption of milk. For example, there is a large market for non-dairy milk targeted to individuals who present with milk (e.g., lactose) allergies, people to subscribe to vegan diets, and individuals who are concerned with the presence of antibiotics, pesticides and hormones commonly found in commercial milk. It is only recently that companies are developing synthetic milk that has the same composition as cow's milk without antibiotics, pesticides and hormones. Perfect Day is a California start-up company that is developing milk proteins derived from genetically engineered yeast [7]. They expect to be on the market sometime in 2020.

Meat

Red meat is long been established as an important dietary source of protein and essential nutrients including iron, zinc and vitamin B12. According to some scholars (e.g., Leslie Aiello) human beings starting to eat calorie-dense meat and marrow instead of the low-quality plant diet of apes, to provide enough extra energy at each meal to help fuel a bigger brain. Digesting a higher quality diet and less bulky plant fiber have allowed these humans to have much smaller guts. The energy freed up as a result of smaller guts could be used by the greedy brain [8]. Today, the rise in "fast foods" that rely on meat consumption has perpetuated throughout our culture. Yet, while meat is an important staple of western diets, there are many reports that describe health risks associated with meat consumption including increased risk of cancer and heart disease [9]. Moreover, the huge demand for land required to grow animals for meat has triggered an examination whether alternative methods to produce healthier meat products without animals has resulted in the development of synthetic or cloned meat and poultry.

Applying Synthetic Biotechnologies to Make Animal-Free Milk

The food tech company Perfect Day is creating an "animal-free milk" product made from proteins derived from yeast fermentation [10]. Using a Genetically Modified (GM) strain of yeast (nicknamed Buttercup) as the biological machinery, this company will produce a milk product that retains the taste and texture of cow's milk. While the proteins in this milk (such as casein and whey) product will be synthesized from yeast, the fats in this product will be obtained from vegetable sources. Moreover, sugars and minerals will be added to the product to improve its

texture and taste. Although Perfect Day's milk will be lactose-free and vegan, the casein and whey contained in this product are still potentially antigenic and would restrict its consumption by people with milk allergies. One of the main motivations to produce milk in yeast is that the taste of plant derived non-dairy milk is not the same as animal generated milk [11].

Applying New Biotechnologies to Make Animal-Free Meats

The basic composition of meat includes water, protein and amino acids, minerals, fats and fatty acids, vitamins and other bioactive components, and small quantities of carbohydrates. While there are many companies focusing on making tasty plant-derived meats and poultry, there are various considerations that have stimulated companies to create animal-free meats that have the same composition and cooking qualities (e.g., the ability to barbecue) as animal derived meats. Stem cell biotechnologies are being employed to create animal-free meats that are referred to as "clean meat", "cloned meat" or "synthetic meat" [12]. In creating animal-free meats, a small sample of muscle is carefully removed, via a syringe, from a living cow's neck muscle without harming the animal. Muscle precursor cells, called myosatellite cells, are then separated from other cells in the extracted tissue and are grown *in vitro* in a bioreactor. These myosatellite cells proliferate and eventually differentiate to generate muscle fibers that form the essential muscle components of animal-derived meat. A similar stem cell process is being applied to generate cloned poultry meats from chickens [13].

The possibility of growing animal-free meat within an industrial setting has long captured the imagination of many people. Winston Churchill suggested in 1931: "We shall escape the absurdity of growing a whole chicken in order to eat the breast or wing, by growing these parts separately under a suitable medium" [14]. In 2002, scientists from NASA reported the need to develop viable means of supplying safe, healthy, nutritious food to Space voyagers on long journeys [15]. They developed a method to culture in the laboratory adult dorsal abdominal skeletal muscle from goldfish as a source to generate crude "meat" explants that resembled fresh fish filets. They hoped the method of growing fish filets in the laboratory could serve as a renewable food source for human space travel. Unfortunately, cultured goldfish fillets never caught on [16].

In 2008, People for the Ethical Treatment of Animals (PETA) offered a $1 million prize to the first company to bring lab-grown chicken meat to consumers by 2012 [17]. Even a 2-year extension from the original deadline of 2012 wasn't enough to lure applicants for PETA's prize. Even though their $1 million prize expired in 2014, PETA continued to fund research at the University of Missouri to develop methods to produce cloned meat [18]. In November 2009, scientists from the Netherlands announced they had managed to grow meat in the laboratory using the cells from a live pig. The project, was backed by a sausage manufacturer and a 2-million-pound grant from the Dutch government [19]. Time magazine declared cultured meat production to be one of the 50 breakthrough ideas of 2009. Since then, over 30 laboratories from around the world have announced that they are working on cultured meat research. The commercial potential of such meats is evident as Tyson Ventures, the venture capital arm of Tyson Foods, Inc., invested an undisclosed amount in Memphis Meats, a developer of cultured meat processed through animal cells [20]. In addition, the cost of producing one hamburger has dropped significantly. The price of the first lab-grown beef burger, created by Mosa Meats, was equivalent to about $1.2 million per pound, retail. Now, lab-grown hamburger runs for about $11.36 per pound [21].

Advantages in The Production of Synthetic Milk, Cloned Meat and Poultry

The potential advantages to cultured meat and milk include environmental benefits [22], health benefits [23], and marketing benefits (such as animal welfare by killing fewer animals to produce meat) [24].

Environmental Advantages: Environmentally, the production of animal-free milk requires 98% less water, 91% less land, and 65% less energy than milk produced in a typical dairy farm. The production of animal-free milk will also significantly reduce greenhouse gas emissions, as the cows in dairy farms are responsible for roughly 3 percent of global greenhouse gas emissions each year [25].

Traditional methods to produce meat from livestock also requires large amounts of cattle, pigs, and chickens that take up about 30 percent of the planet's ice-free land and can wreak incredible environmental damage. Cattle and pigs generate large amounts of methane and waste that pollute the atmosphere, rivers, and streams. Moving meat production from the farm to industrial bioreactors could mean a significantly greener industry by lowering methane production and animal waste as well as utilizing significantly less land, water, and energy [26].

Health Benefits [27]: It is reported that organic milk contains 50% more omega-3 fatty acids than conventionally produced products and also contains less saturated fat than non-organic. One advantage of producing cultured milk is the capacity to manipulate the product's flavor, fatty acid composition, fat content and ratio of saturated to poly-unsaturated fatty acids through the composition of the culture medium or co-culturing with other cell types. Furthermore, health aspects of the milk can be enhanced by adding factors like certain types of vitamins to the culture medium which might have an advantageous effect on the health [28].

The dairy industry has long advertised its products as an excellent source of nutrition. Dairy products provide a rich source of many nutrients including protein, calcium, magnesium, and

vitamins A and B. Calcium is one of the key nutrients believed to be responsible for the positive findings between dairy and prevention of weight gain.

During the last decade, more than a dozen studies found that higher intakes of milk or dairy calcium may help in preventing weight gain and/or changes in body composition among adolescents [29]. However, other studies show no relationship between dairy consumption and body weight or body fat composition. This has raised a considerable controversy about whether or not the amount or level of dairy intake is associated with loss or gain of body weight or fat. One study [8] reported that dairy intake to be significantly associated with body growth in an adolescent population. In addition, this study found total dairy intake to be positively associated with fat mass, and overall BMI, but only in boys. Many more studies are needed to scientifically assess the benefits and risks of milk free diets.

There are many reports that emphasize the health benefits of diets that exclude meat or poultry consumption. However, very few studies rigorously evaluate and compare the health outcomes of people on omnivorous, vegetarian, and vegan diets as distinct experimental groups. In general, people on vegan diets have lower blood pressure, and lower fasting triacylglycerol and glucose concentrations than omnivorous subjects, as well as a biochemical profile found to be cardio-protective and diabetes protective. Vegetarian diets are also reported to improve insulin resistance, lower diabetes risk, and lower cardiometabolic disease risk [30]. Some research suggests that vegetarian diets, especially vegan diets, are associated with lower Bone Mineral Density (BMD), but this does not appear to be clinically significant [23]. The problem with many of these studies is that they only compared the health outcomes of vegans to omnivores, and not to individuals who are vegetarian. While there are reports of the health risks associated with meat [31], other reports [32] suggest that diets which allow moderate amounts of animal products may be as protective against disease as vegan diets. For example, a pooled analysis of five prospective cohort studies, involving 76,000 subjects, found that both vegetarians and those who followed a "prudent" diet allowing small amounts of red meat benefited from a reduced risk of coronary heart disease and type 2 diabetes [33]. Another health advantage to producing cloned poultry in bioreactors instead of using live birds is that the reduction in chicken farms would lower the risk of viral epidemics (avian flu) that can spread via chickens [34].

Natural meat is reported to contain some cancer-causing agents. In muscle tissue, heme iron is found primarily as the oxygen-binding protein, myoglobin. Heme iron can cause DNA damage and induce the formation of N-nitroso compounds, some of which are potent carcinogens. A study that followed nearly 200,000 post-menopausal women found that the amount of heme iron in their diet was positively associated with an increased risk of breast cancer [35]. Other studies show connections between heme iron intake and various forms of cancer [36]. According to its producers, lab-cultured beef or pork can be made completely free of heme iron. But because lab-grown meat lacks a circulatory system, it's kept in a very high-oxygen environment, which has the unintended consequence of reducing cellular myoglobin expression resulting is less iron.

Marketing Advantages: There are many individuals across the globe who are concerned with animal cruelty issues associated with farm-based productions of meat and poultry [37]. Cloning meat in bioreactors would reduce the number of animals that must be sacrificed and reduce the potential for animal cruelty. In addition, there may be a significant marketing advantage that the public would find cloned meat tastier and a better "meat' to barbecue over plant-derived meats. Finally, this technology can be applied to create a new market of exotic cultured meats or even meat from rare or endangered animals.

Americans love hamburgers and chicken wings. 50 billion hamburgers are sold in America each year and one billion chicken wings are consumed on Super Bowl Sunday alone. Recent polls report [38] that 96% of Americans eat fast food meats. Moreover, Americans love the taste of real meat and believe plant made meat does not adequately mimic the taste, convenience, and barbecuing potential of animal-based meats. Surveys about meat consumption report that:

1. Eight in ten Americans report eating at fast-food restaurants at least monthly, with almost half saying they eat fast food at least weekly [39]. About two-thirds of Americans report that their doctors share the benefits of a healthy diet with them, yet, they prefer to eat fast food regularly.
2. Americans tend to ignore nutritional information while eating out: Less than half of Americans say that they pay a "great deal" or a "fair amount" of attention to nutritional information on menus.

One outcome from surveys is that not only do Americans enjoy eating out at fast food establishments but the health of foods offered in these establishments is not a major concern. Thus, one might conclude that given an equal pricing, the majority of Americans would favor animal-free meat over plant-derived meats. A 2017 online survey of US participants [40] concluded that most respondents were willing to try cloned meat, but only one third were definitely or probably willing to eat it regularly or as a replacement for farm-produced meat. This survey also showed that vegetarians and vegans were more likely to perceive potential benefits of cloned meat compared to farmed meat but were less likely to try it than meat eaters. The main concerns of those who did not want to try cloned meat were an anticipated high price, limited taste, and a concern that the product was unnatural and therefore unhealthy. The results of the survey showed that people in the USA are likely to try cloned meat, but few believed that it

would replace farmed meat in their diet.

Challenges in the Production of Cloned Meat and Poultry

There are various scientific challenges in culturing myosatellite cells from animal muscle to produced cloned meat. First, myosatellite cells are a rare cell within muscles and display limited regenerative potential. Second, these cells are prone to malignant transformation in long term culture. While the health risks of consuming such malignant cells have not been extensively studied [41], there is concern whether the consumer might want to avoid consuming "bovine cancer cells" that may be present in cloned meat. To minimize malignant transformation by these cells, companies producing cloned meat will use stem cells that are constantly re-harvested from new cows in the production system. Third, at this time, maintaining these cells in culture requires the use of animal (bovine) serum, in the media that nourishes these cells. Bovine serum is a costly component that may not be readily acceptable to potential consumers because obtaining animal serum requires the sacrifice of many fetal calves. The use of animal serum in culturing meat cells also questions whether this process will really reduce animal use and suffering. However, scientists are rapidly trying to develop serum-free media to avoid the use of animal serum in producing cloned meat. Finally, producers of cloned meats must demonstrate that these meats have the same taste, texture, juiciness, and barbecue potential as animal-derived meat.

Companies Gearing Up to Produce Animal-Free Meat and Poultry

The technology to produced laboratory-grown meat is moving along at a rapid pace. Mosa Meats [42], based on the research and technology developed by Mark Post. produced the first commercial cloned hamburger for culinary experts to taste. In 2013, Dr. Post made headline news around the world for producing the world's first lab-grown burger that was cooked and eaten at a news conference in London. The burger was made from real meat grown in a lab (using 20,000 strips of muscle tissue) at a cost of $325,000. As stated above, a recent ABC News interview with Post reported that the cost of a cloned burger has dramatically dropped to just over $11 for a burger ($80 per kilogram of meat) [43].

The first cloned burger was cooked by Chef Richard McGeown of Couch's Great House Restaurant in Cornwall and tasted by Hanni Ruetzler, a food critic and food researcher at the Future Food Studio in London. Ruetzler described the experience as follows: "There is really a bite to it, there is quite some flavor with the browning. I know there is no fat in it so I didn't really know how juicy it would be, but there is quite some intense taste; it's close to meat, it's not that juicy, but the consistency is perfect. This is meat to me… It's really something to bite on and I think the look is quite similar."

Since the first public trial, several other startups have made advances in the field. Memphis Meats [44], a Silicon Valley startup founded by a cardiologist, launched a video in February 2016 showcasing its cultured beef meatball. In March 2017, it showcased chicken tenders and duck a-l'orange, the first cultured poultry-based foods shown to the public [45]. At a tasting event in March of 2017, people who tasted lab-grown chicken strips swear it tastes just like chicken [46].

Other major corporate players in this market include:

1. An Israeli company, SuperMeat [47], ran a viral crowdfunding campaign in 2016 to produce cloned chicken meat.
2. Perfect Day Foods: a startup focused on animal-free dairy products.
3. Shojinmeat: a Japanese biohacker community that is developing cloned meat.
4. Finless Foods, a California-based biotech startup, takes a small sample of cells from a living marine animal and cultured them in a brewery-like environment in the shape of a fish fillet. The process takes several months to complete, with a product that features the same texture and appearance of real fish meat. Unlike commercial fish farming, Finless Foods' process requires no antibiotics, hormones or harmful chemicals and delivers only the part of the fish that consumers want to eat [48].
5. New Harvest, Good Food Institute, and the Modern Ag Foundation: Non-profit research institutes dedicated to the field of cellular agriculture, focused on the development of animal-free eggs, milk, meat, and more non-animal products.

Ethical, Cultural/Religious Issues with Animal-Free Meat and Milk

As mentioned above, animal cruelty is one of the most discussed ethical issues in meat production. Despite many governmental safeguards, there is still evidence of animal cruelty in generating meat from cows, pigs and chickens [49]. Many articles [50] have been written that discusses the issue of animal cruelty in the meat industry. In principle, the production of cloned meat should help resolve this ethical concern.

Aside from animal cruelty, there are several other ethical concerns that emerge from producing cloned meat. First, potential consumers may be repulsed by cloned meat on grounds that it is unnatural or some perversion of biology. Second, the public may be concerned whether cloned meat is harmful to human health. Although there is much confusion in the USA about what is considered a healthy diet [51], the publication of cookbooks on the use of cloned meat is a first step in marketing a product to the public. Two recent books [The "*In Vitro* Meat Cookbook", by Koert Mensvoort, and Hendrik-Jen-Grievink, and "*In vitro* meat: protein for twelve billion?" by Adam May, and Jean Fleming],

may alleviate some of the health concerns and fears of consuming cloned meat. Another final issue is how vegetarians and vegans will view cloned meat compared to plant-derived? The answer to this question will depend on the reasons why people choose to eliminate meat from their diets. If the reasons are to avoid cruelty to animals, then cloned meat would be a valid alternative to animal-derived meat. However, if the concerns are related to the perceived negative health effects of consuming animal products, then cloned meat will still require the use of animal cells and may not be a solution for vegetarians.

Religious Concerns Related to Cloned Meat

Two major religions of the world - Islam and Judaism - have restrictions on the consumption of meat and dairy products. In Islam, animal meat cannot be porcine in origin and must be obtained from appropriate animals ("Halal"- which means lawful or permissible). In Judaism, animal meat must be obtained from "Kosher animals" (primarily domesticated animals, such as cow, sheep, goat, deer, and buffalo). In Halal- and Kosher-certified facilities, meat must be obtained from animals that are sacrificed using a slaughtering procedure designed to reduce animal suffering.

There are fundamental religious principles regarding Halal meats. For example, the majority of Islamic scholar's view that it is permissible to eat horse meat. It was narrated from Jaabir ibn 'Abd-Allaah that he said: "On the day of Khaybar, the Messenger of Allaah forbade eating the flesh of domestic donkeys, but he granted a concession with regard to horses." Narrated by al-Bukhaari, 3982; Muslim, 1941. In contrast, the following living organisms are not Halal-acceptable [52]:

1. Insects considered ugly or filthy such as worms, lice, flies, etc.
2. Animals with fangs such as tigers, lions, cats etc,
3. Birds that have talons with which they catch their prey such as owls, eagles, etc.
4. Animals which Islam encourages to kill such as scorpions, centipedes, rats etc.
5. Dogs
6. Animals which Islam forbids to kill such as bees etc.
7. Animals which have toxins, poisons or produce ill effects when eaten such as some fish etc.
8. Amphibian animals such as crocodiles, turtles, frogs etc.
9. Lawful animals not slaughtered according to Islamic rites. (Fish is exempt from slaughtering).
10. Carrion or dead animals [53].

There also are at least two differences regarding the views of Muslim and Jewish scholars regarding cloned meat. First, as stated above there are many "non-Kosher" animals that are acceptable as Halal-certified. Second, observant Jews will not consume or cook milk and meat products together. Both Jewish and Islamic legal scholars have stated that cloned meat would only be permissible for consumption if the cloned meat were produced from muscle-derived stem cells that were obtained from either a Kosher or Halal-accepted animal and certainly not porcine-derived. Second, the muscle sample must be obtained from the animal after slaughter because according to both Jewish and Islamic law one is not allowed to extract a tissue from a live animal for food consumption. Third, the maintenance of cultured meat in the bioreactors currently requires the cells to be grown in media containing bovine serum. Bovine serum used in traditional bioreactors are not typically obtained from Kosher or Halal certified companies and might render the meat as non-Kosher or non-Halal certified. In the Jewish tradition, for example, the biblical verse that forbids the consumption of blood might apply to the consumption of animal serum as well. In the Islamic tradition (according to Sharee'ah), the presence of blood in meat is one reason why it is forbidden to eat meat that has not been slaughtered. Sharee'ah directs that the slaughtered animal should be drained of blood as completely as possible, and that is because of the extreme spiritual harm that would result from eating its blood. Currently, there are no publications that address the specific religious issue of using bovine serum to produce cloned meat. However, in the future, the religious concerns of using bovine serum in cloning meat may not be germane for either religious Jews or Muslims because commercial companies are trying to eliminate the use of bovine serum for maintaining stem cells in bioreactors. For example, SuperMeat has developed a technology to grow and maintain poultry-derived stem cells in the absence of animal serum in order to generate kosher cloned chicken meat.

Regarding Jewish law, there are some additional complications regarding the use of kosher cloned meat. As mentioned above Jewish law prohibits the consumption or cooking of meat and milk products together. Thus, the question is whether cloned meat is viewed as animal meat or another byproduct of the animal (similar to milk) that does not carry a meat status? I propose that since the biological origin and source of animal meat is myosatellite cells then any product subsequently generated from these stem cells will retain their original status as essential components of muscle or meat. Thus, many Jewish scholars would classify cloned meat as being technically considered equal to animal meat and would prohibit its consumption with any dairy products [54]. This ruling differs from the general Jewish legal/religious ruling of gelatin. Gelatin is not considered to possess the status of meat because it is derived from bones or skin and not flesh. Thus, many Jewish authorities permit the consumption of gelatin with dairy products.

Cloned poultry (obtained from stem cells of a chicken and turkey) will also require that the bird source be a Kosher species

and that the cells be obtained after the bird is slaughtered. However, cloned chicken or turkey meat differs from cloned beef meat in one important aspect. From a Jewish perspective, meat obtained from poultry is biblically classified as a non-meat product. It is only through a rabbinical decree that meat obtained from poultry is not cooked or consumed with any dairy products. Because this is a rabbinical decree, some current Jewish authorities would not institute another rabbinical prohibition above and beyond an existing rabbinical decree. Thus, using myosatellite cells to generate chicken meat may render this product as a non-meat product or parve [40,55]. Such a rabbinical ruling would allow the production of cheeseburgers using cloned chicken meat and real cheese.

Currently, there may consenting views from either Jewish or Islamic scholars regarding the religious issues of cloned meat discussed in the article. However, history has taught us that only when a product actually hits the public market, will religious scholars carefully review how the product is made and re-assess the religious status of such a product. Thus, we expect that until cloned meat or yeast-derived milk products appears on the market shelf, there will be various opinions issued by Jewish and Islamic scholars. If history is a guide, I would predict that many Jewish and Islamic scholars might readily accept cloned meat derived from cows or poultry, rather than pigs, that has been properly slaughtered as being a religiously permitted food. However, it is much more difficult to predict how the vegan communities will accept cloned meat as compared to plant-derived meat. I imagine that marketing, texture, and taste - along with the health risks, health benefits, and affordability - will be important considerations in how the vegan community will accept or reject the incorporation of cloned meat and poultry into their diets.

Regarding milk-derived from yeast, the major religious issue will be from the Jewish religious community. I predict that such products will be classified within Jewish law as a non-dairy product because it is not obtained from a mammalian source. Thus, milk-derived from yeast will be no different than soymilk, rice milk or almond milk and will be considered non-dairy (or Parve) in Jewish Law - allowing it to be consumed with any animal-derived meats or poultry.

Conclusions

There is no doubt that the use of gene editing and stem cell biotechnologies will continue to have a huge cultural, environmental, and financial impact on the food industry. Besides using these technologies to generate meat and milk, many companies are using genetic methodologies to produce wine products [56], opioids [57], cheeses [58], and gelatin [59]. The practical challenges for this industry are four-fold. Will their products be healthier than animal-derived products, less expensive, mimic the taste of the animal-derived counterparts, and will the production of these products have a positive impact on resources and the environment? From an ethical perspective, vegan, Jewish, and Muslim communities will have to decide under what conditions and provisions will cloned meat be a viable food entity.

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