THE DEVELOP CULTURED MEAT FROM BLACK-BONED CHICKEN EMBRYONIC STEM CELL

PATCHAREE PROMTAN

DOCTOR OF PHILOSOPHY (ANIMAL SCIENCE) IN ANIMAL SCIENCE

MAEJO UNIVERSITY

2023

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A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (ANIMAL SCIENCE)
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บทคัดย่อ

การศึกษานี้มีวัตถุประสงค์เพื่อศึกษาประสิทธิภาพการเจริญเติบโตของสเต็มเซลล์จากตัวอ่อนไก่กระดูกดำสำหรับการพัฒนาเนื้อสังเคราะห์ที่เพาะเลี้ยงจากสเต็มเซลล์ตัวอ่อนไก่กระดูกดำในอนาคต วางแผนการทดลองแบบสุ่มสมบูรณ์ (Completely Randomized Design; CRD) ในการวิเคราะห์ข้อมูลทางสถิติ การคัดเลือกและเพาะเลี้ยงเซลล์ต้นกำเนิดจากตัวอ่อนไก่กระดูกดำจากไข่ที่ปฏิสนธิแล้วจะใช้อาหารในการเพาะเลี้ยงเซลล์ที่มีซีรั่มที่แตกต่างกัน ได้แก่ Fetal Bovine; FBS (T1), ไก่เชิงการค้า; SCK (T2), ไก่ประดู่หางดำ; PDC (T3),ไก่กระดูกดำ; BBC (T4) สเต็มเซลล์จากตัวอ่อนของไก่กระดูกดำถูกเพาะเลี้ยงในตู้บ่มมีสภาพแวดล้อมที่อุณหภูมิ 37.0 °C มี CO_2 5% ทำการวัดการเจริญเติบโตของเซลล์ตัวอ่อนไก่กระดูกดำด้วยเครื่องสเปกโตรโฟโตมิเตอร์ที่ความยาวคลื่น 450 นาโนเมตร พบว่า T4 มีการเจริญเติบโตเร็วกว่ากลุ่มอื่นหลังจากเพาะเลี้ยงสเต็มเซลล์ 2 ชั่วโมง T3 มีการเจริญเติบโตเร็วกว่ากลุ่มอื่นระหว่าง 4 ถึง 12 ชั่วโมง T4 มีการเจริญเติบโตมากกว่ากลุ่มอื่นใน 24 ชั่วโมง T2 มีการเจริญเติบโตมากกว่ากลุ่มอื่นที่ 48 - 192 ชั่วโมง ในขณะที่ T4 มีการเจริญเติบโตมากกว่ากลุ่มอื่นระหว่าง 216 และ 240 ชั่วโมง ($P < 0.05$) เมื่อนับเซลล์ตัวอ่อนพบว่า T4 มีจำนวนและอัตราการเจริญเติบโตสูงกว่ากลุ่มอื่นอย่างมีนัยสำคัญ ($P < 0.05$) เมื่อทำการเปรียบเทียบปริมาณโปรตีนของเนื้อไก่กระดูกดำที่เลี้ยงในห้องปฏิบัติการกับเนื้อไก่ที่เลี้ยงปกติตามธรรมชาติด้วยวิธีการไนโตรเจนคอมบัสชัน พบว่า T4 มีโปรตีนมากกว่ากลุ่มอื่นอย่างมีนัยสำคัญทางสถิติ ($P < 0.05$) รองลงมาคือ T2 T3 และ T1 ตามลำดับ เซลล์เนื้อไก่เชิงการค้ามีพื้นที่หน้าตัด (4,214.49 μm^2) และ เส้นผ่านศูนย์กลาง (99.43 μm.) มากกว่ากลุ่มอื่นอย่างมีนัยสำคัญ ($P < 0.01$) แต่ในทางเดียวกันเมื่อเปรียบเทียบพื้นที่หน้าตัดและเส้นผ่านศูนย์กลางของเซลล์เนื้อไก่สังเคราะห์กับเซลล์เนื้อไก่พื้นเมืองพบว่า ไม่มีความแตกต่างกัน ($P > 0.05$) เนื่องจากเนื้อสัตว์เพาะเลี้ยงมีแนวโน้มโปรตีนใกล้เคียงกับเนื้อไก่ เทียบเคียงได้กับเนื้อไก่จริง และลักษณะของเนื้อที่เลี้ยงไม่ต่างจากเซลล์ไก่ทั่วไป ทั้งนี้ การเพาะเซลล์ต้นกำเนิดจากตัวอ่อนไก่กระดูกดำเพื่อผลิตเนื้อสัตว์สังเคราะห์จำเป็นต้องใช้อาหารเลี้ยงเซลล์ที่มีซีรั่มไก่กระดูกดำเป็นส่วนประกอบ

คำสำคัญ : เนื้อสังเคราะห์, สเต็มเซลล์ตัวอ่อน, ไก่กระดูกดำ

Title	THE DEVELOP CULTURED MEAT FROM BLACK-BONED CHICKEN EMBRYONIC STEM CELL
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ABSTRACT

The objective of this study was to investigate the growing performance of black-boned chicken embryonic stem cells to create cultured meat for the future development of cultured meat from black-boned chicken embryonic stem cells. The data were statistically analyzed by a Completely randomized design (CRD). Isolation and culture of black-boned chicken embryonic stem cells from fertilized eggs. Different serums, Fetal Bovine; FBS (T1), Commercial chicken; SCK (T2), Pradu Hang Dam chicken; PDC (T3), Black-Boned chicken; BBC (T4) were allocated to the treatments. Black-boned chicken embryonic stem cells were cultured at 37.0°C in a humidified environment of 5% CO_2. The growth of black-boned chicken ES cells was measured at 450 nm. absorbance. Found that, T4 grew faster than the other groups after cultivating stem cells for 2 hr. T3 grew faster than any other group between 4 and 12 hr. T4 had grown more than any other group in 24 hours. T2 grew more than the other groups between 48 - 192 hr., while T4 had greater growth between 216 and 240 hr. ($P < 0.05$). When counting ES cells, T4 had a much greater number and growth rate than the other groups ($P < 0.05$). Compared the protein content of laboratory-cultured black-bone chicken meat to wild-farmed chicken meat by nitrogen combustion method. T4 was found to have significantly more protein than the other groups ($P < 0.05$), followed by T2, T3, and T1, respectively. The median cross-section area (4,214.49 μm^2) and diameter (99.43 μm.) of the SCK muscle were significantly higher ($P < 0.01$) than those of the other groups. But at the same time, the cross-sectional size of the culture muscle cells was not different from that of the

two types of native chicken muscle cells (P > 0.05). Because cultured meat is tending to be similar in protein to chicken meat and comparable to real chicken meat and characteristics of the cultured meat are not different from normal chicken cells. Finally, the cultivation of black-boned chicken embryonic stem cells for culture meat necessitates the use of a media containing black-boned chicken serum.

Keywords : cultured meat, embryonic stem cell, black-boned chicken

ACKNOWLEDGEMENTS

Words cannot express my gratitude to my professor and the chair of my committee for their invaluable patience and feedback. I also could not have undertaken this journey without my defense committee, who generously provided knowledge and expertise. Additionally, I would like to thank The Animal Husbandry Association of Thailand under The Royal Patronage of H.R.H Princess Maha Chakri Sirindhorn for funding my research dissertation from Dr. Wiboon Lapjatupon of The Inteqc Feed Company Limited Fund, poultry farm Faculty of Animal Science and Technology, Lab of Livestock Reproductive Biotechnology, and Innovation and Upgrading the production of Fa Luang black bone chicken into high-value poultry products for Northern Thailand food Valley project.

I am also grateful to my classmates and cohort members, especially my office mates, for their editing help, late-night feedback sessions, and moral support. Thanks, should also go to the librarians, research assistants, and study participants from the university, who impacted and inspired me.

Lastly, I would be remiss in not mentioning my family, especially my parents. Their belief in me has kept my spirits and motivation high during this process.

Patcharee Promtan

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LIST OF ABBREVIATIONS

&	=	And
%	=	Percentage
$	=	Dollars
°C	=	Degree celsius
BBC	=	Black-Boned chicken serum
BC	=	Blastodermal cells
CO_2	=	Carbon dioxide
CP	=	Crude Protein
CRD	=	Completely randomized design
DM	=	Dry matter
EDTA	=	Ethylenediaminetetraacetic acid
EE	=	Ether extract
ESC	=	Embryonic stem cells
FBS	=	Fetal bovine serum
HCL	=	Hydrochloric acid
hr.	=	Hours
IMDM	=	Iscov's Modified Dulbecco's Medium
Kcal	=	Kilocalories
KCl	=	Potassium chloride
Kg	=	Kilogram
M	=	Molarity

ME	=	Metabolizable energy
ml.	=	Millilitre
N	=	Normality
P-value	=	Probability value
PBS	=	Phosphate Buffered Saline
PDC	=	Pradu Hang Dum chicken serum
RH	=	Relative humidity
SCK	=	Chicken serum
SEM	=	Standard error of mean
ul.	=	Microliter
µm.	=	Micrometer
μm^2	=	Square micrometer

CHAPTER 1 INTRODUCTION

According to projections for a recovery in meat output in China, Brazil, Vietnam, the United States, and the European Union, world meat production would increase by 2.20% to 346 million tonnes in 2021. But decreased in Australia, Argentina, and the Philippines. China's predicted increase in meat production matches the trend of increasing output across all meat categories. Large-scale investments in biosecurity and improving the meat supply chain are particularly important for pork. The sale of services corresponds to the effectiveness of COVID-19 immunization, better sanitation, and government aid to the cattle industry in the COVID-19 crisis, as well as market stabilization initiatives. The meat value chain remains tense owing to ongoing market limitations (COVID-19), rising feed prices, a shortage of animals, or a drought. Conversely, poultry meat has the highest demand for production of meat. The global meat products trade is estimated to reach 42 million tonnes (carcass weight equivalent) in 2021 due to continued meat shortages and domestic production limits. The researchers were interested in black-boned chicken, which is a native chicken, popularly raised and consumed by indigenous peoples. It has an important role in farms, small farmers, and local people. Black-boned chicken is a healthy source of protein as well as reduces fatigue, anxiety, metabolism stimulation, control blood sugar and blood pressure, and helps to strengthen the immune system (Li et al., 2012). Currently, the shortage of high-protein food sources, especially chicken, ranks first among land meats (swine and beef providing protein 2nd and 3rd respectively), is low in fat. Chicken is one of the fast-yielding economic animals. Because it takes a shorter time to raise than other economic animals. It also has a high feed conversion ratio (Alexander et al., 2020) and good disease resistance, resulting in higher production and consumption of chicken than other meat types. The impact of the poultry farming and raising industry on the community on air pollution and noise from animal farms is air pollution. There is an issue with the bad odor. The problem of dust and flies Including air pollutants emission by farm animals that are harmful to human and

animal health, such as hydrogen sulfide gas, ammonia, carbon dioxide, carbon monoxide, methane, volatile organic compounds, particulate matter, including feed dust, wool, dry manure, litter, microorganisms, pollen, insect dust, and farm burning fumes. Plant raw materials used as feed, etc., including excreted toxins (endotoxin) and noise pollution, particularly in communities with animal farms. Which counts the days until such problems become increasingly severe (Wilailuck, 2012). Global warming is the result of these impacts, certain groups of individuals have begun to agitate against eating meat to prevent global warming and promote animal slaughter. As a result, most people are unable to resist meat-based foods. But to cut greenhouse gas emissions while meeting consumer demand for meat. Green meat, created in a laboratory food, has been developed by scientists. Using biotechnology and culinary understanding, laboratory meat has just been marketed internationally. Which is not the processing of plant proteins. Rather, it's actual meat grown from stem cells, which are specialized cells that can proliferate or morph into any cell. They are then cultivated in labs using a proper culture media to generate meat for cooking, known as cultured meat. The benefits of culture meat include the ability to minimize greenhouse gas emissions while also reducing the usage of soil and water resources. It will also limit future human exposure to antibiotics, stimulants, and certain diseases from meat if the cost of making culture meat is reduced further. Culture meat will aid in the alleviation of food scarcity. Especially meat that is difficult to avoid soon. It is anticipated that by 2050, there will be more than 9.7 billion people due to the growing world population. More than double the amount of land and resources needed for farming. When employing traditional meat production methods. There will be insufficient space on the planet to grow animals (Tummy, 2019).

Protein is essential for people of all ages. Whether or not, childhood protein is required to help the body develop. Tissues and hormones are strengthened. People of working age require protein to restore worn out portions. Take good care of your skin and your immune system. and provide freshness throughout the day It also helps to keep your tummy full during rush hour. Somebody who stretches muscles loses more protein than usual since they are working so hard. As a result,

protein must be reinforced to develop and mend the muscles that have been worn down by exercise. It's especially for the elderly as they age the elderly's digestion is impaired. Protein that is easily digested is essential for the elderly. Protein must be absorbed fast to compensate for muscular degeneration caused by aging. It also aids the body's recovery from illness and tiredness. Among these are immunity boosters (Faibis, 2016). According to a current demographic survey in Thailand, there are 66,186,727 people (Thanakhom, 2021). According to the projection, Thailand will become an aging society with a population in the future. People over the age of 65 will account for 14% (~9,266,142) and 20% (~13,237,346) of the total population in 2022 and 2032, respectively. There are many more.

Production of cultured meat based on lab-produced, black-boned chicken. The development of cultured meat production system based on black bone chicken will be able to solve the problem of meat shortage cleanliness and germ-free control of meat better than meat from the market. Safe from antibiotics and hormones in animal husbandry will remain on the meat and can also improve some nutrients to be higher as needed, such as adding collagen, fat, omega 3, etc., for another choice of healthy people and the elderly (Del Favero et al., 2012). It also helps address food shortages, reduce pollution, reduce space, and resources in meat production above.

Objectives

To develop cultured meat from black-boned chicken embryonic stem cell.

Hypotheses

Production of cultured meat based on lab-produced, black-boned chicken embryonic stem cells

Benefits expected

1. Can produce cultured meat from black-bone chicken stem cells.
2. Capable of developing a synthetic meat manufacturing system based on black-boned chicken.

3. Black-bone chicken manufactured from cultured meat is healthy. It may be served with a flavor and texture comparable to that of normally bred black-boned chicken.
4. In the incubation phase of black-boned chicken eggs, stem cells of early developing embryos can be preserved.

Anticipated outcomes

Changes that occur because of the growth of cultured meat from broad-spectrum chicken bone stem cells. Long-term accomplishments that emerge because of improvements in outcomes because of the process of engagement activities and having an impact pathway to push towards producing an impact through both direct and indirect communication will help farmers earn more money because of both purposeful and unexpected events, including the economy. There is enough food for customers, especially excellent quality food that is simple to eat for individuals who care about their health, the elderly, and the influence on society and the environment. will acquire safe synthetic meat Reduce pollutants from conventional chicken farming.

CHAPTER 2 LITERATURE REVIEW

Black-boned chicken

Black-boned chicken is a native chicken that is popularly farmed and consumed in upper northern areas such as Chiang Mai, Chiang Rai, Mae Hong Son, etc. There are guidelines to develop them into economic animals at the small-scale farmer level as well, based on the Chinese idea that boneless chicken, when cooked with Chinese medicine, is more nutritious than chicken or other types of chicken. Even eating black-boned chicken that has not been stewed with Chinese medicine is thought to nourish the body better than eating other types of chicken flesh. As a result, Chinese residents in the North choose to raise black-bone chickens for food and sell them to neighbors or those in need, which will sell for more than double the price of domestic chicken. However, according to their beliefs, most Thai people prefer to utilize black-bone chicken in ceremonies. It is also used to refuel the body. As a result, black-boned chicken became increasingly popular but was not available in the broader market. This is because black-boned chickens are quite rare. There are not enough to meet demand. Including the genuine, black-boned chicken breed It's also difficult to come by.

1. Information about black-boned chicken in general

The term "black-boned chickens" refers to the black characteristics of three parts of the chicken body, namely the skin, meat, and bones, caused by an excessive accumulation of melanin pigment in the tissues. As reported by Smyth Jr. (1990), melanism or fibro melanosis has been connected to the sex-linked id+ gene and the dominant enhancer gene Fm. The combination of these two genes causes black buildup to darken in connective tissue. The black-boned chicken is completely black, and every bone in the body must be black. If the bone is not black and may have other colors such as yellow or white, it is not classified as "real black bone chicken." The skin and muscle may be a dark black color. Gray is not accepted as a fault in the black-boned chicken breed.

This black-boned chicken was created by the Royal Project Foundation to have good development and breed-specific characteristics: nine black regions at the mouth, face, tongue, crest, claws, tibia, skin, bone, and meat (Suchon et al., 2014). This is related to the expression of the fibromyalgia (Fm) gene, which stimulates the growth of black pigment cells stored in bone and skin tissue. Dermal melanin is involved in hyperpigmentation processes (Dorshorst et al., 2010).

Figure 1 Black-boned chicken

2. Breeding of black-boned chickens

Permsak (2004) investigated and chose black-boned chicken breeds originally by evaluating hair color due to the variety of black-boned chicken breeds. However, all three sections must be black: skin, meat, and bones. The study divided black-boned chickens into five species, which are classified as follows:

1) Black-boned chicken with a gray neck and red stripes; F2 chicken with 67.74% black feather color.
2) Golden Black Bone Chicken with 91.66% brown feather color in F2.
3) Golden Bone Gray Chicken with 61.11% black feather color in F2.
4) Black boned chicken with a striped neck, 63.16% black feather color in F2.
5) White boneless chicken or cheese boneless chicken; F2 chicken feather color is all white.

However, different breeds of black-boned chicken need more breeding research. To develop a breed that possesses both the exterior (chicken feather color) and internal characteristics of the three black-boned chickens discussed before, for the advantages of both farming as a pastime for beauty and as a form of Chinese medicine for nourishing the body, which is another way to increase family income.

Nattakarn et al. (2015) chose chicken breeds and other selection criteria from the foundation's existing black-bone chicken flock. At 22 weeks, there were 162 puppies—34 males and 128 females—with 60 pups, 10 males, and 50 females chosen to be utilized as a breeding herd (P0). The following selection criteria will be used for future production of F1 chicks:

1. Weighed selection criteria (growth performance) Choose chickens who are healthier and have a greater body weight than the average of the male and female flocks. Following that, the exterior qualities were chosen using the criteria in Item 2.

2. When considering breed-specific physical characteristics as selection criteria, consider the following five visual characteristics using photographic comparison methods: (Figure 2)

1) Crest and face: The color of the chakra's crest and the face must be black (Figure 2; a1).

2) Legs and Nails: The shin and nail color must be black or gray; white is permitted if the number of chickens is minimal (Figure 2; b1 and b2).

3) Skin: The skin must be black gray in tone (Figure 2; c1).

4) Tongue: The sublingual color must be black, covering more than 2/3 the length of the tongue or more than 50% of the tongue; if white, reject it (Figure 2; d1 d2 d3 d4).

5) Palate: The palette must be black in hue. If there aren't enough hens, it can be gray; if it's white, it must be discarded (Figure 2; e1, e2, and e3).

The dark interior region is made up of flesh and bone. This time, it has not been employed as a selection criterion. As a result of selecting chickens while they were still alive

Figure 2 Characteristic of Black-boned chicken

Source: Natthakarn et al. (2015)

Carnosine and melanine

Carnosine is a dipeptide derivative of the amino acids beta-alanine and histidine, which are found mostly in muscles, brain tissue, and heart muscle. Carnosine may be produced by the body on its own. Carnosine has several physiological and biological properties at the cellular level, such as pH buffering, anti-aging, anti-glycation, anti-inflammation, antioxidation, anti-fatigue, and as a neurotransmitter in skeletal muscle, which contributes to muscular stretching. By controlling muscle protein regulation (Kralik et al., 2010). It also has a relationship with melanin, which is abundant in black-boned chicken. It has anti-aging characteristics as well as several antioxidant properties, including metal scavenging and oxygen scavenging, free radical elimination, and peroxide decomposition (Begum et al., 2005). Also, carnosine helps keep fresh pork and cattle red. The meat's red color is due to carnosine's antioxidant action, which prevents the development of meat methamyoglobin during storage. Carnosine has musculoskeletal system buffering characteristics (pH buffering), may trap carbonyl groups (carbonyl scavenging) that have antioxidant activity, and can slow down cell aging as well as assist mitochondria in creating greater energy, so helping to improve the energy for the cells. Aids in the promotion of learning and memory processes. Prevent Alzheimer's disease-related brain and heart insufficiencies. Aids in the improvement of heartbeat Moreover, it may be used to prevent or cure diabetic problems. Caused by cell inflammation, cataracts, and other factors. It is also particularly effective in promoting muscle healing in athletes and the elderly.

Based on an examination of the quantity of melanin found in the chicken's different organs by Muroya et al. (2000) and Chen et al. (2008) found that, the bony periostcum has more melanin than the organs. Other bodily components are followed by reproductive organs (ovaries and testes), the trachea, and the skin. Carnosine levels may be high in these organs' tissues as well. (**Table 1-2**)

This is because most consumers only consume black-boned chicken for the brisket and thighs. Tian et al. (2007) Carnosine content in black-bone chicken and Plymouth rock bar chicken was studied. HPLC was used to evaluate black-boned

chickens, and all mixed-sex Plymouth Rock chicks aged 7 0 -9 5 days received the same feed and menagment. Carnosine content was determined in the breast and rump portions of black-boned chickens and Plymouth Rock barn chickens. Carnosine levels in black-boned chicken were found to be greater than in Plymouth Rock bar chicken (**Table 2**). Next, Khumpeerawat et al. (2021) study the carnosine content and its correlation to the expression of carnosine-related genes were investigated in Thai native chicken and black-boned chicken breast meat. The carnosine level in each breed and breed group ranged from 428.08 mg/100 g to 553.93 mg/100 g.

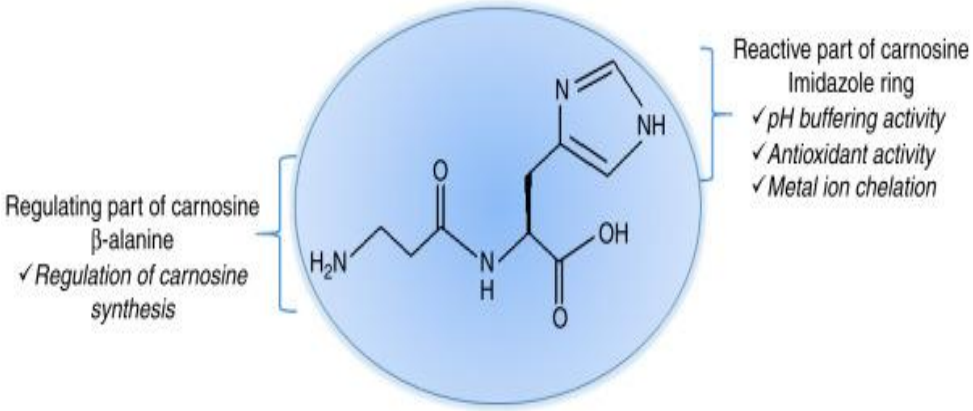

Figure 3 Structure carnosine

Source: Dogru et al. (2018)

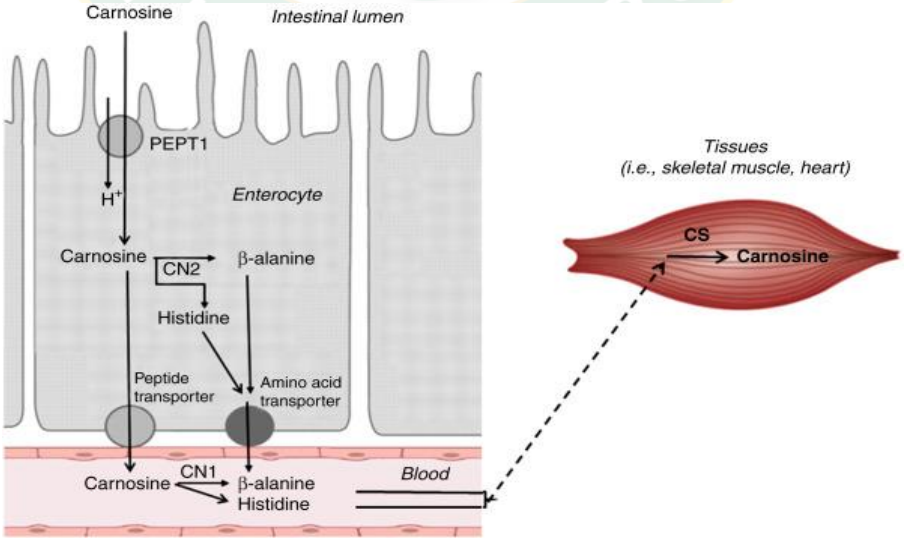

Figure 4 Carnosine in muscles

Source: Dogru et al. (2018)

Table 1 Melanin in the organ tissues of Silky black boned chicken and White leghorn laying hens

organ	The amount of melanin (mg/g)		
	White leghorn laying hens[1/]	black boned chicken[1/]	black boned chicken[2/]
Membrane (femur)	0.27	21.00	21.30
ovaries or testicles	0.14	9.70	10.70
bronchia	0.60	8.60	10.20
skin	0.012	0.944	1.10
intestine	0.053	0.889	NA
heart	0.112	0.124	NA
liver	0.092	0.072	NA
Supracoracoideus	0.009	0.067	NA
breast meat	0.010	0.050	1.00
gizzard	0.046	0.039	NA

NA = No data available

Source: [1/]Muroya et al. (2000), [2/]Chen et al. (2008)

Table 2 Content of carnosine in muscles from Black-Bone Silky Fowl and White Plymouth Rock

Samples	Black-Bone Silky Fowl			White Plymouth Rock		
	Moisture (%)	Content of carnosine (mg/g)		Moisture (%)	Content of carnosine (mg/g)	
		Wet basis	Dry basis		Wet basis	Dry basis
Mixed meat	75.5 ± 0.2	1.1 ± 0.6*	4.5 ± 0.8*	72.1 ± 0.1	0.6 ± 0.4	2.2 ± 0.5
Breast meat	76.1 ± 0.8	1.6 ± 0.3*	6.6 ± 1.1*	75.9 ± 1.1	0.9 ± 0.4	3.9 ± 1.4
Thigh meat	77.9 ± 0.7	0.4 ± 0.1*	2.0 ± 0.2*	77.6 ± 0.9	0.2 ± 0.1	0.9 ± 0.6

Results are presented as means ± SD (n = 10); compared with White Plymouth Rock.

* $P < 0.01$

Source: Tian et al. (2007)

Situation and demand for meat consumption

The global meat products trade is estimated to reach 4 2 million tonnes (carcass weight equivalent) in 2021, nearly unchanged from 2020. Production increase in the cattle and poultry sector is expected, according to projections virtually all. The pork trade is projected to decrease. China is anticipated to boost the global meat trade overall. Due to continued meat shortages and domestic production limits, a total purchase of almost 1 1 million tonnes of meat was made. Furthermore, international meat prices grew from January to May, showing increasing demand for meat imports, particularly from East Asia and the Middle East despite slowing global growth show in **Table 3** (FAO, 2021). The average global chicken consumption rate is 14.80 kg/person/year followed by pigs and beef, 11.10 and 6.40 kg/person/year, respectively (**Figure 5**). Most are in the form of 1) chilled chicken, 2) frozen chicken, and 3) processed or frozen cooked/seasoned chicken. Each product has a different production process. Moreover, the global demand for broiler consumption is likely to continue to increase show in **Figure 6** (MOC, 2020)

Table 3 World meat market immediately

WORLD BALANCE	2019	2020 estim	2021 f'cast	Change: 2021 over 2020
	million tonnes (carcass weight equivalent)			%
Production	**337.2**	**338.1**	**345.6**	**2.2**
Bovine meat	72.4	71.6	72.4	1.2
Poultry meat	131.6	133.4	135.2	1.3
Pig meat	110.1	109.8	114.4	4.2
Ovine meat	16.2	16.2	16.5	1.3
Trade	**36.6**	**41.7**	**41.9**	**0.4**
Bovine meat	11.3	11.8	12.0	1.1
Poultry meat	14.2	15.4	15.6	0.9
Pig meat	9.6	12.9	12.8	-0.6
Ovine meat	1.0	1.1	1.1	-0.8

Source: FAO (2021)

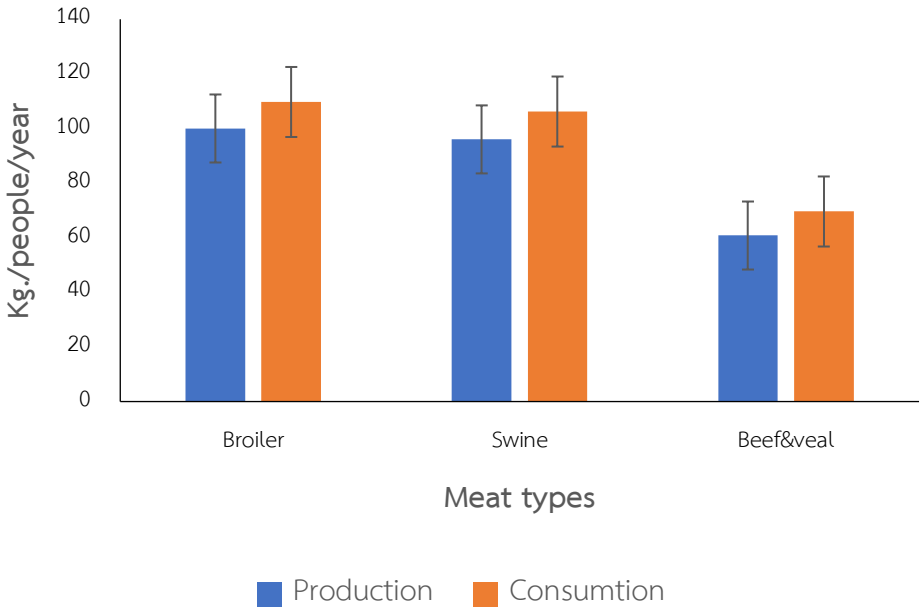

Figure 5 World top terrestrial animal production and consumption in 2020

Source: Adapted from USDA (2020)

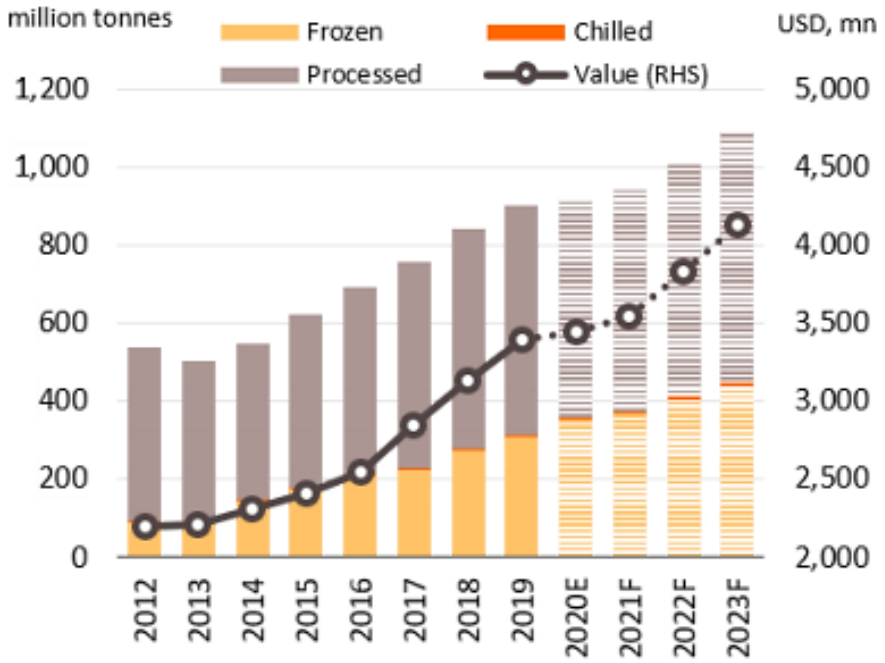

Figure 6 Exports of thai chicken products

Source: Adapted from Sowcharoensuk (2020)

Dutch scientists first introduced cultured meat to the public in 2013. Only 2-3 years later, scientists from around the world have discovered it in the form of beef burgers, which cost as much as 15 million baht per kilogram (Charoen, 2018) and provide impetus for scientists throughout the world. The cost of culture meat is decreasing, and it is believed that it will be commercially available in the near future. In addition to culture beef, culture pork, poultry, and tuna are being developed as consumer alternatives (Pawita, 2020). Post and a group of scientists from Mosa meat, one of the businesses creating culture meat, highlight the benefits of culture meat, which is meat produced without the use of animals. Emissions of greenhouse gases are reduced. Decrease the utilization of soil and water resources. It also decreases the consumption of antibiotics, stimulants, and certain diseases from meat to people, and in the future, if the cost of making culture meat is decreased even more, artificial meat will address the issue of food scarcity. Particularly meat, which will be impossible to avoid in the near future. Because of the world's expanding population, it is anticipated that by 2050, there will be more than 9.7 billion people to feed. The amount of land and resources needed to raise animals has more than doubled if standard meat production procedures are used. The planet will not have enough area to rear animals. (Tummy, 2019)

In 2022, Thailand will become a completely aged society (Aged Society: population over 65 years old, accounting for more than 14% of total population). After that, Thailand is anticipated to become a completely aged society (Super-Aged Society: population over 65 years old (more than 20% of total population)) in 2032. The growing population of the elderly has contributed to the rise of the market for products and services for the aged. Currently, the market for products and services for the elderly is projected to be worth at least 900 billion baht each year, or around one-third of the country's overall GDP. Even though the market value of products and services for the elderly continues to rise. However, it is not without difficulties. This is because most Thai senior persons, accounting for more than 95% of the total elderly population, have poor incomes (less than 300,000 baht per year on average) and rely solely on their children for income (Kasikorn Research Center, 2021). According to Kasikorn Research Center, customers will begin to focus more on

quality and product standards following the COVID-19 issue, as market conditions change. Furthermore, trading partners might propose various types of trade measures to defend commerce. It will also serve as a stimulus for entrepreneurs to adapt to the future of food goods and respond to the New Normal lifestyle, which is more conscious of environmental hygiene, the environment, society, and good governance on the consumer side. Functional food, organic food, and alternative protein from plants or insects are goods with considerable commercial potential. Food spices and herbs that are ready-to-cook or ready-to-eat (Kasikorn Research Center, 2021). Even though laboratory-produced culture meat is becoming increasingly popular, food manufacturers face several hurdles. Specifically, the cost is because laboratory-produced meat is substantially more expensive than plant-based alternatives. Furthermore, increasing competition will reduce the cost of making culture meat in the lab, which recently reached $478,993 per kg. (Approximately 14.4 million baht) to $8,164 per kilogram (about 246,300 baht) in 2016 and is predicted. Although it will only cost 14.5 USD (approximately 437 baht) in 2020, the price is still more than the market average for chicken (H_2O, 2020). Therefore, the researcher is producing of cultured meat based on lab-produced, black-boned chicken. The development of cultured meat production system based on black bone chicken will be able to solve the problem of meat shortage cleanliness and germ-free control of meat better than meat from the market. Safe from antibiotics and hormones in animal husbandry will remain on the meat and can also improve some nutrients to be higher as needed, such as adding collagen, fat, omega 3, etc., for another choice of healthy people and the elderly (Del Favero et al., 2012). It also helps address food shortages, reduce pollution, reduce space, and resources in meat production above.

Chicken embryo fertilization and development

Fertilization is the combination of sperm cells from a man and an egg (egg or ovum) from a female. Although polyspemic sperm can penetrate the vitelline membrane, only one can combine with the egg's pronucleus; the rest is known as surplus sperm. Supernumerary sperms are eliminated four to five hours after fertilization, which occurs in the fallopian tubes infundibulum (Petitte et al., 1997).

Embryonic development before incubation

Syngamy occurs when the pronuclei of the sperm cell and the egg unite, resulting in the formation of a single embryonic cell as the yolk flows into the isthmus (**Figure 7**). The embryo will then proceed to divide. continually When the egg moves from the infundibulum into the uterus in about 1 hour, cell division will range from 8 to 256 cells, requiring around 4 hours since the hen's body temperature is 41 ^{o}C to increase embryo cell division. with only one storey built first When the egg enters the vagina, two layers of cells, the ectoderm and endoderm, create a plate-shaped, pale white blastodisc next to the yolk. In the hen, the egg takes 24 to 27 hours. Chicken blastodisc is approximately 3 mm in diameter and contains 40,000 to 60,000 cells outside the hen and smaller (Petitte et al., 1990; Watanabe et al., 1992).

Embryonic development during incubation

When the temperature is raised, the embryo stops developing. Incubating an egg causes the embryo to develop in the blastodisc area, which retreats on top of the yolk. They are around 3 mm in diameter (Burley and Vadehra, 1989; Etches et al., 1996). The central cell is transparent, with a pellucida region surrounding the yolk and an opaca area between the yolk and the pellucida area. When eggs hatch, a region known as the pellucida differentiates. It is a two-layer tissue, with the epiblast on top and the hypoblast on the bottom. The hypoblast layer typically forms into an extraembryonic membrane layer. From stages XI to XIII, the hypoblast occurs, and by stage XIV, the top epiblast thickens, a primitive streak forms, and three layers are evident. The streak whitens the hypoblast in the anterior direction before merging into an endodermal layer; this location is known as the germinal crescent. The mesoderm, the third cell layer, forms between the ectoderm and endoderm layers, bridging the gap between the first two cells. Blastocoels differentiate from the three cell layers: 1) the ectoderm gives rise to skin, feathers, beaks, claws, and the rectal system; 2) the mesoderm is responsible for the development of bone, muscles, blood, reproductive organs, and excretory organs; and 3) the endoderm gives rise to the embryo. secretory and digestive systems (Prathom, 1997)

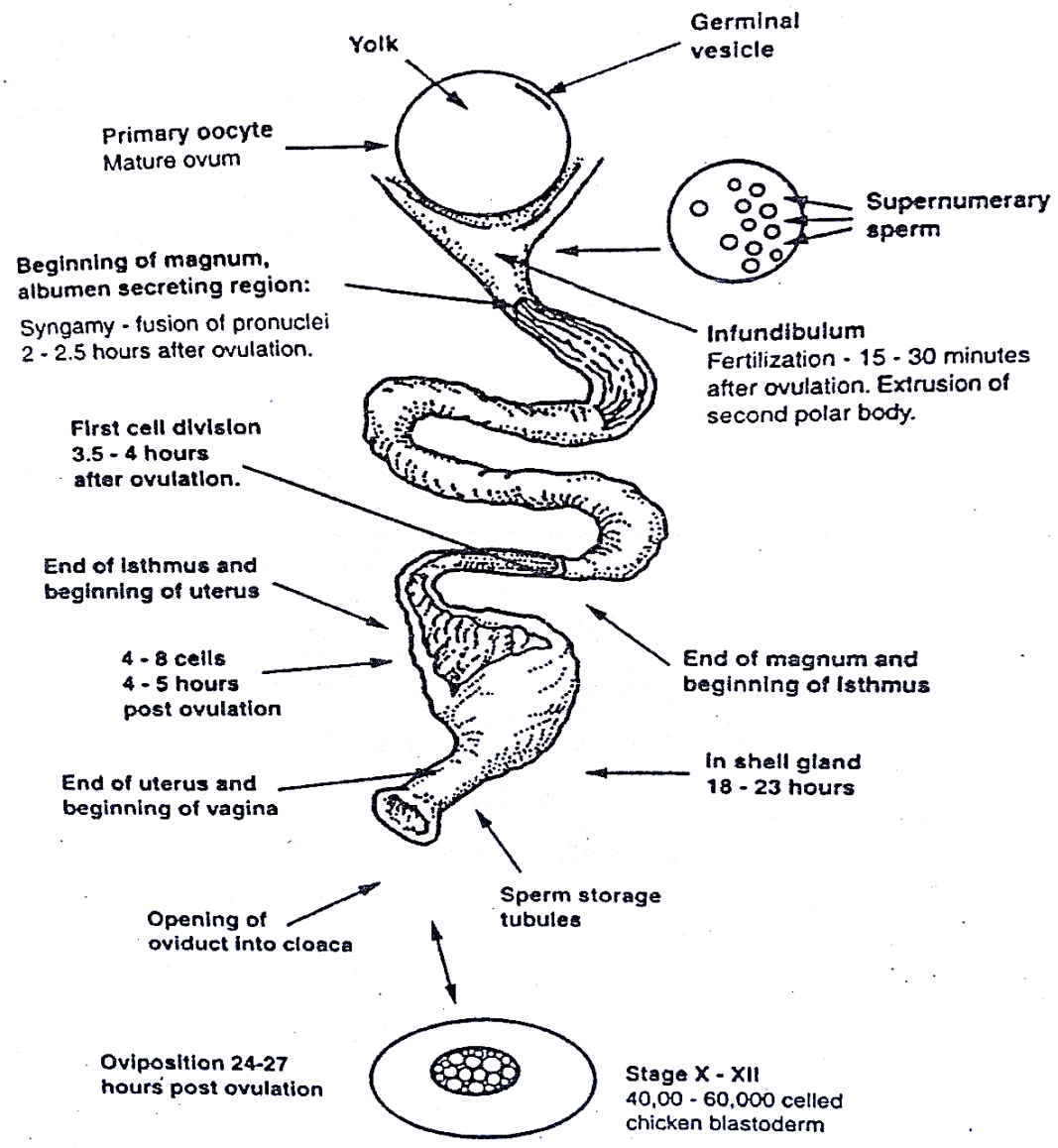

Figure 7 The development of pre-hatching embryo stage

Source: Renaville and Bury (2001)

Stem cells

Stem cells are progenitor cells that can transform into many other types of cells, primarily embryonic cells, and are found in several animal organs. Completely developed to classify stem cells, there are three primary categories. To begin, totipotent stem cells are stem cells, which are embryonic cells derived from a newly fertilized egg and could turn into any type of cell, including embryos. Pluripotent stem cells are embryonic cells that may develop into a full embryo but cannot become part of the placenta; both totipotent and pluripotent stem cells are referred to as embryonic stem (ES) cells. Third, multipotent stem cells are stem cells found in some organs that can develop into different types of cells in that organ, such as haematopoietic stem cells found in bone marrow that can differentiate into white blood cells, red blood cells, and platelets (McKay et al. 2000). (**Figure 8**)

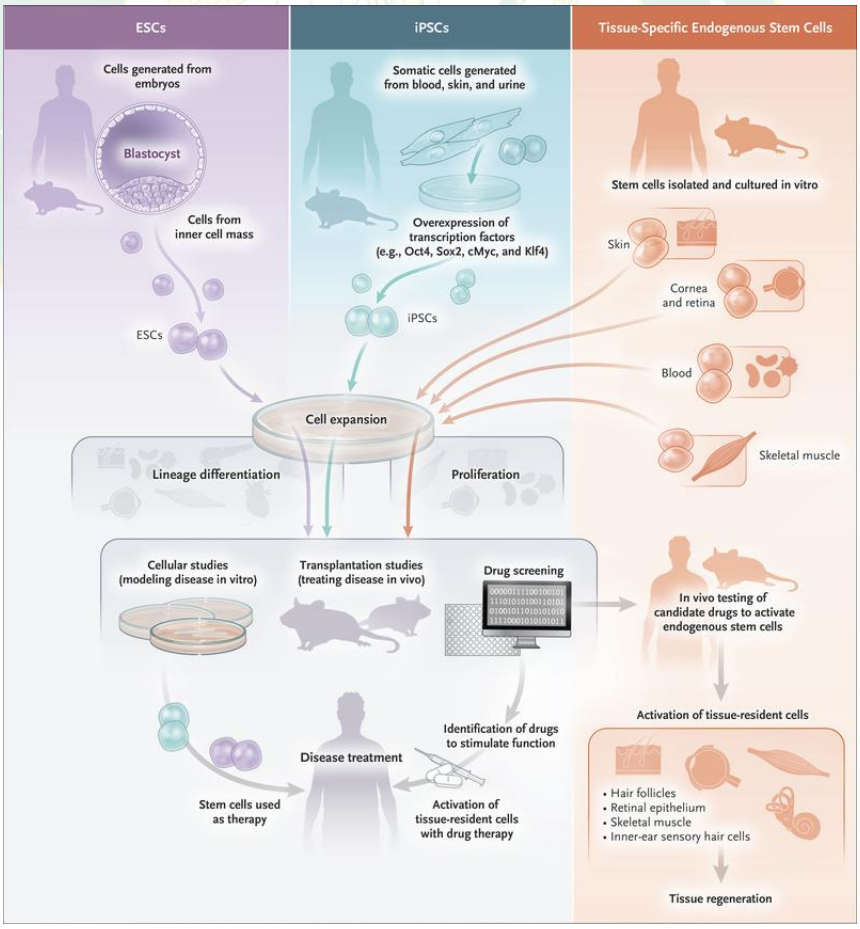

Figure 8 Stem cells type

Source: Blau and Daley (2019)

Embryonic Stem Cell (ESC)

The first ESC discovery was achieved by investigating embryonic carcinoma (ECC) cells, which are immature cells like ESCs. In culture, the undifferentiated cells survived and multiplied. When mouse ECC was injected into the embryonic spaces of a recipient mouse blastocyst, the cells changed and grew into malignant tissues in receptor animals (Evans and Kaufman, 1981). However, ESC differs from ECC in that it can persist in culture media for longer periods of time and has a higher organoid capacity (Matin, 1981). These findings provide crucial information for clinical stem cell culture research. ES cells are totipotent and pluripotent stem cells discovered in early embryonic development. Because of their simplicity, pluripotent stem cells are being explored nowadays. In mammals, ESCs are cells from the inner cell mass of blastocyst embryos (Evans and Kaufman, 1981); in chickens, ESCs are blastodermal cells from stage X embryos (Eyal-Giladi and Kochav, 1976). In 1996, Pain and researchers cultivated chicken embryonic stem cells. Electronic microscopy was used to characterize chicken embryonic stem cells in terms of differentiation, growth, and morphology, and it was found that those characteristics demonstrated the stem cell nature of chicken embryonic stem cells and that cESCs could be cultured for an extended period without exhibiting evidence of a growth crisis or non-aging cells, including the necessity for a nurse cell that boosts growth factors and cytokines. Furthermore, cESCs may be genetically modified utilizing a variety of vectors, including simple expression vectors (Pain et al., 1999). If stage X embryonic stem cells (BC) were not cultivated in the blastodermal cells (BC) fraction after differentiation, gene-attached vectors can be seen within the microscopic morphology of various embryonic stem cells (Acloque et al., 2001). When grown at this stage, they can develop into progenitor germ cells and other cells (Lavoir et al., 2006).

Primordial Germ Cell (PGC)

PGC are embryonic stem cells that can turn into gametes as animals mature. Four PGCs can be discovered during embryonic development in poultry: 1) At embryonic stage X, at the middle of the blastodisc; 2) as the embryo grows in the

following stage, the PGC migrates to the hypoblast at stages XI to XIII, the germinal crescent, with the PGC continuing anteriorly and laterally at the 18th hour of incubation (Eyal-Giladi and Kochav, 1976); 3) PGC migrates to the circulatory system during embryonic circulatory development. and increasingly frequent during stages 13 to 14, around 50 hours of incubation (Ginsburg and Eyal-Giladi, 1986; Muniesa and Dominguez, 1990); and 4) After 72 hours, the germinal ridge begins to divide into germ cell precursors, from which the PGC differentiates into oogonia or spermatogonia to produce eggs and sperm. (Swit et al., 1914; Fujimoto et al., 1976; Eyal-Giladi et al., 1981; Ginsberg and Eyal-Giladi, 1986)

Presently, there are three techniques for characterizing PGC:

1. PGC classification based on cell shape features Scanning electron microscopy was used to gain morphological characterization and intracellular composition (SEM). PGCs were discovered to be spherical in shape, with the cytoplasm coated with microvilli (Matsumura and England, 1993), with an average diameter of 14–20 cm. microns, whereas PGCs isolated from the circulatory system are 12 m in diameter and round nuclei are 8–9 m in diameter. They are distinguished by an eccentric nucleus (Wentworth et al., 1989). The cytoplasm of PGC has a considerable quantity of glycogen. Until it is four days old, the embryo is in its early stages of development. (Wentworth et al, 1989; Ginburg, 1986)

2. Whole-mount immunohistochemistry or the expression of antigenic markers on the cell surface of undifferentiated PGC It employs antibodies that are specific to carbohydrate and protein structural locations.

3. Alkaline phosphatase staining technique identification because the surface area of PGC has a high glycogen content, resulting in cell surface media (Wentworth et al, 1989; Chang et al., 1995a; Peitte et al, 1999; Park and Han, 2000). As a result, alkaline phosphatase labeling has been utilized to identify mouse PGC cells (Matsui et al., 1992) and chicken ES cells (Pain et al., 1996).

The Study of Embryonic Stem Cells

Poultry embryos are effective methods for studying developmental biology and stem cells (Stern, 2005) and are largely utilized to investigate stem cell biology. They are the only non-mammalian animal species capable of producing embryonic stem cells and germ cells. Stabilized chicken embryonic stem cells (cESC) and chicken embryonic germ cells (cEG) are classed as pluripotent stem cells (Petitte et al., 2004), although cultivated poultry embryo germ cells may contribute to pathogens in vitro (Petitte et al., 2004). (van de Lavoir et al., 2006a). The limitation of embryonic stem cells affecting germ cell culture can be explained by early germ cell determination and in vitro downregulation of germ cell potential (Lavial et al., 2009). The Vasa homologue (Cvh) gene may be important for germ cell specification and the identification of chicken primordial germ cells (cPGCs) capable of entering the germ cell stage. For embryonic stem cell cultivation, chicken embryonic stem cells are often isolated from blastodermal stage X cells. with several sitting cells Growth factors and cytokines are also present (Aubel and Pain, 2013). Similarly, embryonic stem cells may be maintained by mouse embryonic stem cells (mESC). Using leukemia inhibitory factor (LIF) as a parent cell in an undifferentiated state (Horiuchi et al., 2006), Current embryonic stem cell isolation methods extract pluripotent embryonic cells from pluripotent embryonic stages. In vitro, both primordial germ cells (PGCs) and spermatogonial stem cells (SSCs) may develop into neuroblast-like adipocytes and osteoblasts and display gene markers that are comparable (Li et al., 2010; Jung et al., 2007). (Li et al., 2010; Jung et al., 2007). So, embryonic stem cells from chickens can be isolated from embryos and cultivated in the lab. which are collected from diverse sources at various stages of embryonic development and may verify the fact that it is a pluripotent embryonic cell by producing an embryo and splitting into a single cell, making it equivalent to mammalian stem cells. This serves as a model for researching stem cell biology, etc. The preservation of progenitor stem cells from avian embryos that require genetic preservation is a very effective procedure (Kang et al., 2008). The possibilities of success are determined by the cultural system. It is critical for cells to multiply. Using laboratory animals to increase the number of cultivated cells in the laboratory while minimizing the use of embryos

to obtain stem cells for research is not only ethical. Methods for growing poultry stem cells without chaperone cells and with chaperone cells are now available. Systems that do not require chaperone cells, on the other hand, can only be grown for a limited time. Long-term continuous culturing of stem cells in co-culture with peer cells demonstrated cell growth and preservation success. In poultry stem cell cultures, several types of caretaker cells have been used, including Sandoz inbred mouse-derived Thioguanine resistant and Ouabain-resistant (STO), mouse fibroblast feeder (MEF), and chicken embryonic fibroblast (CEF) (Van). (De Lavoir et al., 2006; Jung et al., 2005; Wang et al., 2010). Using heterogeneous animal cells as caretaker cells, on the other hand, increases the chance of progenitor cells becoming contaminated with secretions from the heterogeneous caretaker cells. And the activation of those molecules may change the characteristics of stem cells. Using the same cell type as the stem cells can help prevent contamination (Naito et al., 2015). soft as well.

Over the last two decades, great progress has been achieved in the discovery, selection, and manipulation of stem cells. For meat production, several kinds of stem cells are being studied. Myoblast cells, or progenitor cells, are the most important. They are mature tissue stem cells. It is, in fact, a cell in charge of developing new muscles to replace worn-out ones. However, maintaining the replicative condition in cell culture has proven problematic. Stem cells develop more easily into adult myotubes and myofibrils. As a result, it is chosen as the primary cell source for the development of skeletal muscle tissue (Collins et al., 2007). In 2009, Ezashi et al. described that iPSCs are developed cells such as fibroblasts that have been rendered pluripotent by stable transfection with a particular set of four transcription factors (Oct4, Sox2, KLF4, and c-Myc) that drive the embryonic gene expression program in cells (Takahashi and Yamanaka, 2006). Stem cells can be employed as basic cells for in vitro meat synthesis. will distinguish the product Furthermore, the biochemical makeup of meat may be changed to make it a healthier diet or a specialized product, such as by increasing the content of polyunsaturated fatty acids or elements that are good for the human body.

Mammalian cell culture on a large scale was pioneered in the 1950s. Since advances in cell media, incubators, and serum production, for example (Post, 2012).

Embryonic stem cell assays

The development of embryonic stem cells may be studied in a variety of ways, ranging from visual or microscopic early characterization to in-depth genetic analysis (**Figure 9**). Endoscopy is used to examine growth characteristics and other changes. Most stem cells are seen using various types of cameras.

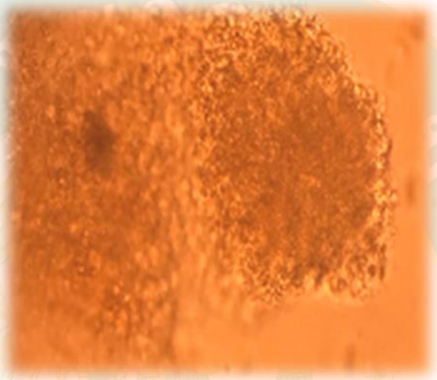

Figure 9 Isolation of chicken embryonic stem cell (cESC)

Electronic microscope

Electronic microscopes are utilized to characterize chicken embryonic stem cells in terms of dynamics, proliferation, and ESC shape. Nucleocytoplasmic ratio and a distinguishing characteristic of the nucleolus (Figures 10A, 10B), whose cell size ranges from 10 to 12 m. It has a huge nucleus that measures 5 - 7 m. There is a dense mitochondrial network in the cytoplasm (arrow, Figure 10B) as well as a big Golgi apparatus embedded in the reticulum cytoplasm, which includes a significant number of ribosomes (Figure 10C). The clusters (5 cells in Figure 10A) are inextricably linked. The intercellular gap is relatively small, measuring around 0.1–0.4 m. When the distance between neighboring cells can be calculated, gap joints and tight joints are examples of unusual construction. Only a high-magnification microscope can reveal it. These characteristics suggest that chicken embryonic stem cells are stem cells.

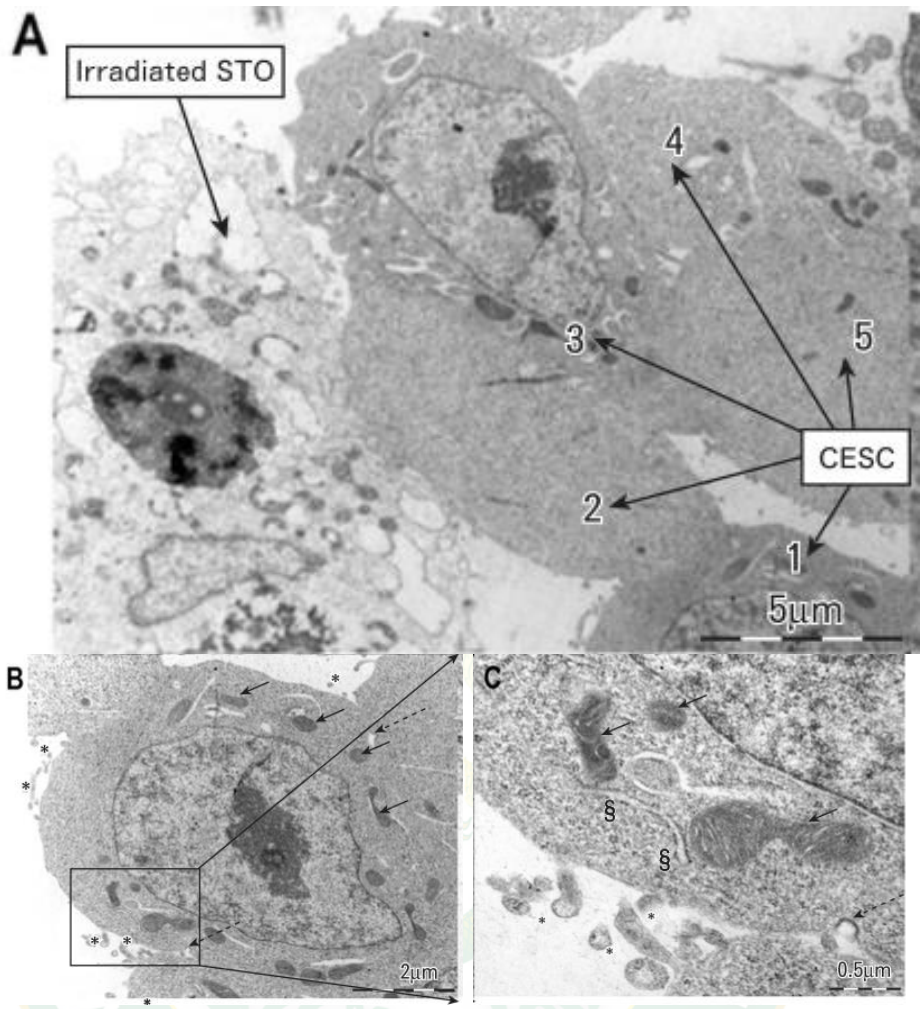

Figure 10 Electron microscopy analysis of proliferating cESC

Source: Lavial et al. (2006)

Biochemical characterization of embryonic stem cell markers utilizing fluorescent cameras

Embryonic stem cells from many species include markers that may identify species-specific traits. Most antibodies to SSEA1, SSEA3, and SSEA4 were grown and compared to mouse progenitor cells. and have been demonstrated to detect stem cells from many species. The cross-species interaction of these antibodies with chicken cells produced from B cells was documented by Lavial and Pain (2010). Lastoderm is cultivated. The interactions with SSEA1 and SSEA3 were particular to chicken cells, as mESC activated SSEA1 but not SSEA4, whereas hESC

did not activate SSEA1 but did activate SSEA4 (**Table 4**), alkaline phosphatase (**Figure 11**), and fluorescent markers (**Figure 12**). In addition to examining the properties of mammalian stem cells, cESCs may be grown for an extended period without evidence of a growth crisis or non-aging cells.

Table 4 Main features of embryonic stem cells (ESCs) from different species

	mESC	hESC	cESC
AP	++	++	++
SSEA1	++	-	++
SSEA3	-	-	++
SSEA4	-	++	-
Tert	++	++	++
Oct4	++	++	++
Nanog	++	++	++

Abbreviations: AP, alkaline phosphatase; cESC, chicken embryonic stem cells; hESC, human embryonic stem cells; mESC, mouse embryonic stem cells; SSEA1, SSEA3, SSEA4, stage specific embryonic antigen 1, 3 and 4; Tert, telomerase activity, Oct4 and Nanog gene expression.

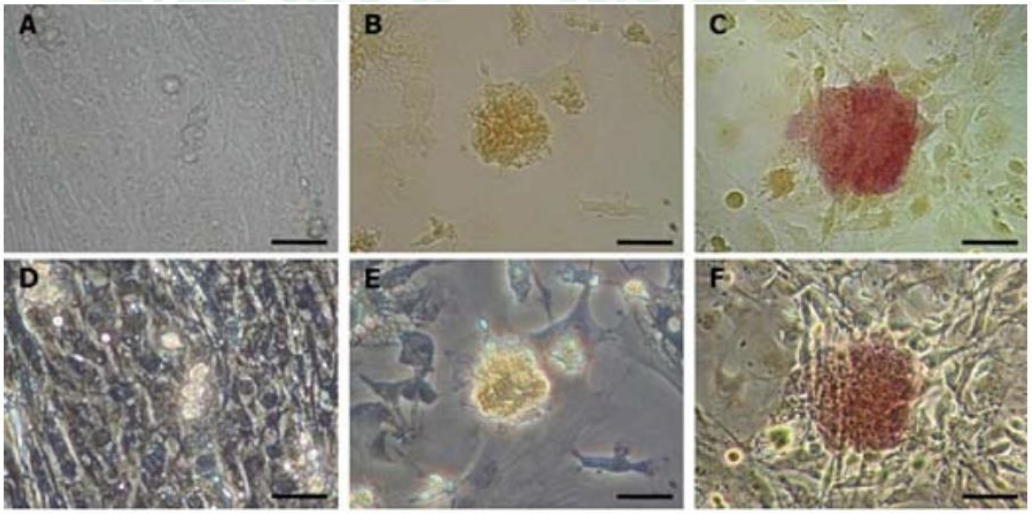

Figure 11 AP activity of colony-forming cells cultured for different periods (6 to 10 days, mESC); (A–C) Inverted microscope image and (D–F) phase contrast image, Bar = 50 μm.

Source: Jung et al. (2007)

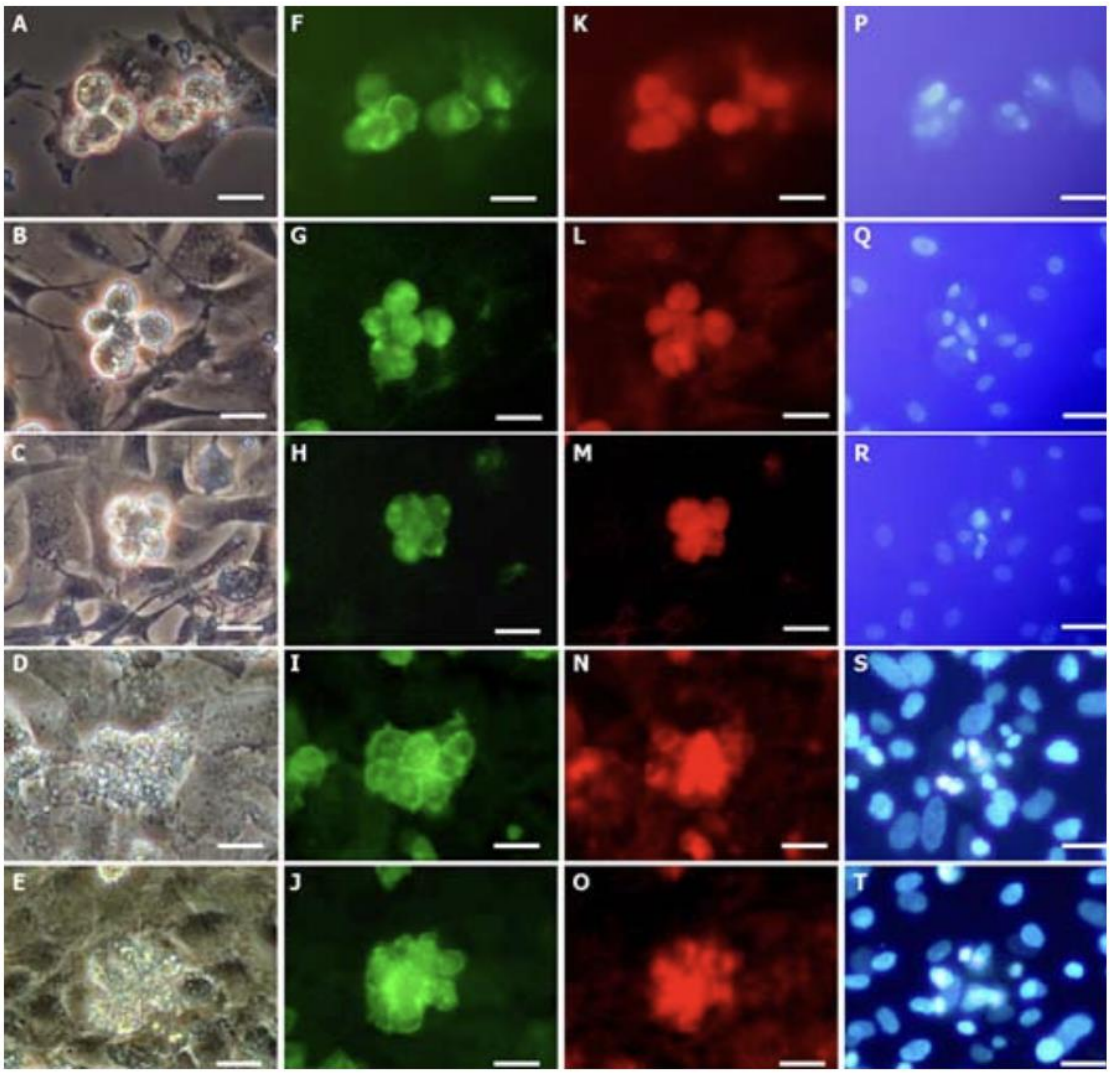

Figure 12 Characterization of chicken colony forming cells; (A–E) phase contrast image for double immunostaining of anti-SSEA1 (K), anti-SSEA3 (L), anti-SSEA4 (M), anti-ITGA6 (N), or anti-ITGB1 antibodies (O) with FITC-conjugated STA (second column; F–J) and DAPI staining (fourth column; P–T). Bar = 25 μm.

Source: Jung et al. (2007)

Genetic characteristics

The genetic coding of the embryos of interest was examined for gene expression using reverse transcriptase-polymerase chain reaction (RT-PCR). The RT-PCR data were subsequently evaluated in embryonic stem cells using

polyacrylamide gel electrophoresis (PAGE), i.e., fatty acid addition, to assess the genetic code modifications and neighboring genes. Using molecular markers to investigate the GF58 gene variation in the genetic code of chicken progenitor germ cells, etc. (**Figures 13–14**)

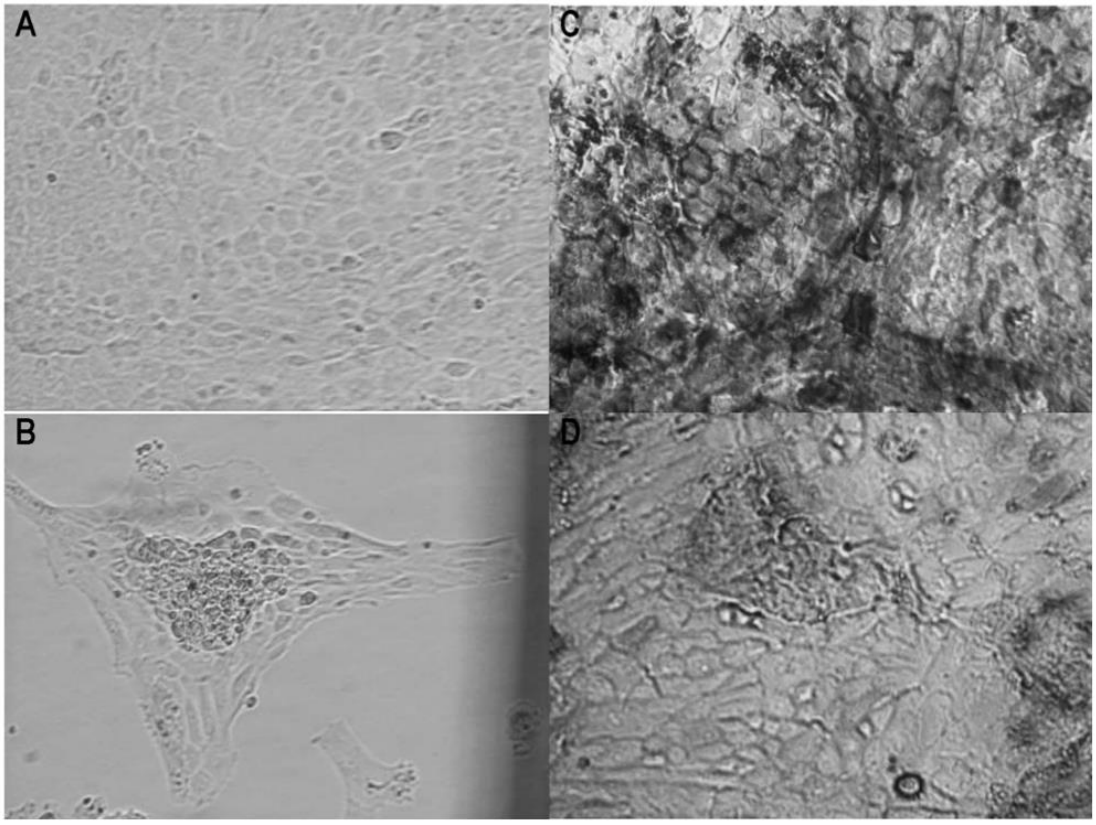

Figure 13 Slowly proliferating GF58 cells. GF58 cells were obtained from in vitro culture of CBC in specific conditions as described in Material & Methods. These cells can be expanded in monolayer -like stem cells (Figure 13A) and proliferate in small aggregates when plated at low density (Figure 13B). GF58 cells exhibit an endogenous phosphatase alkaline activity (Figure 13C) which is lost when the cells are treated for 72 hours with retinoic acid at 10-7M (Figure 13D)

Source: Lavial et al. (2006)

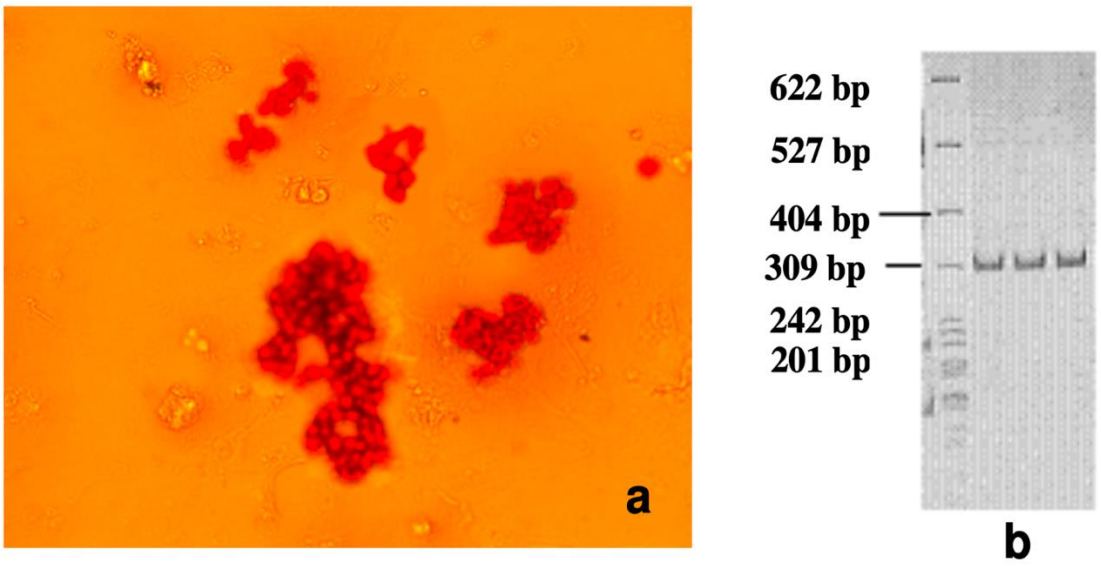

Figure 14 Identification of chicken ES-derived adipocytes. (A) The derived cells were examined directly for lipid granules by oil red O staining on day 21 (200 x); (b) expression of PPAP-γ mRNA in the cells was detected by RT-PCT on day 21 after induction.

Source: Li et al. (2011)

Cultured meat

Cultured meat is created by using mesenchymal stem cells, which could become multipotent stem cells, or cells that can develop into fat cells, bone cells, and other types of cells. muscle cells and cartilage in the spinal cord, mesenchymal stem cells can be detected. Placental umbilical cord, infant teeth, wisdom teeth, and fat cells in the skin layer the primary way is to isolate mesenchymal stem cells from cells of meat-eating animals, such as chicken feathers or bovine muscle. They are taken out and given a medium containing various elements required for the proliferation and induction of mesenchymal stem cells into muscle cells, fat cells, and cartilage cells. Of course, the experiment produces a layer of tissue in which these cells are intermingled. When the cells are cultivated in sufficient quantities, the layers of meat are pulverized and blended with various spices, and then utilized to continue cooking using the stem cell meat concept, removing the need to murder and torture many animals because the method for extracting the animal's cells is

simple and does not harm the animal. It also cuts greenhouse gas emissions from animal farms by up to 90% and significantly decreases land and water pollution from livestock husbandry. However, synthetic meat derived from stem cells is still prohibitively costly. Eat Just, for example, will be distributed under the GOOD Meat trade name in Singapore at prices equal to premium chicken. Due to restricted output and high manufacturing expenses, which include wages, researcher expertise and skills, time expenditures, and costly laboratory equipment. Perhaps in the next few decades. Researchers from across the world have collaborated to create works ranging from the past to the present. and projections for the future (**Figures 15–16**). Stem cell meat may become a favorite of animal lovers and a new addition to the global culinary landscape.

Cor van der Ville found the growth of stem cells in frog tissue in 2004 at a provincial museum in France. Post's team was subsequently successful in creating fake pork in the laboratory (Post et al., 2006), and European researchers were able to establish a process that could generate meat on an industrial scale. "Test-tube hamburger" (Test-tube hamburger) by researchers at the University of Maastrict (University of Maastrict) in Holland by Mark Post in 2013, and the Dutch team is considering creating a meat factory at the village level. Beef, chicken, and pork were all "fake" in 2014. Mosa Meat Company successfully manufactured and sold cultured beef in 2015. However, the price is exceedingly steep, at 95,798 US dollars per kilogram. In 2016, Mosa Meat Co.'s cultured beef manufacturing cost was reduced to $8,164/kg, and it was sold in department shops for the first time. In addition to synthetic beef, various firms, like JUST Incorporated of the United States, are now working to produce additional synthetic meats. which is researching synthetic chicken meat created from chicken stem cells and protein derived from legumes and algae, which presently costs more than $100 per kg, or Finless Foods, which is developing synthetic fish meat, which is projected to be commercially accessible by 2020. Current meat production technologies in laboratories in this work, stem cells from the model animal's muscles were cultivated in a bioreactor at an appropriate temperature and utilizing a plant-derived growth medium. It offers nutrition for the

development of stem cells. This process, however, only yields beef, chicken, pork, and tuna (Choudhury et al., 2020), and each company's research team faces a challenge in producing synthetic burger meat at reduced manufacturing costs, with synthetic beef predicted to cost less than $2 by 2023. (**Figure 16**)

Figure 15 A brief history of fake meat

Source: Walkinshaw (2017)

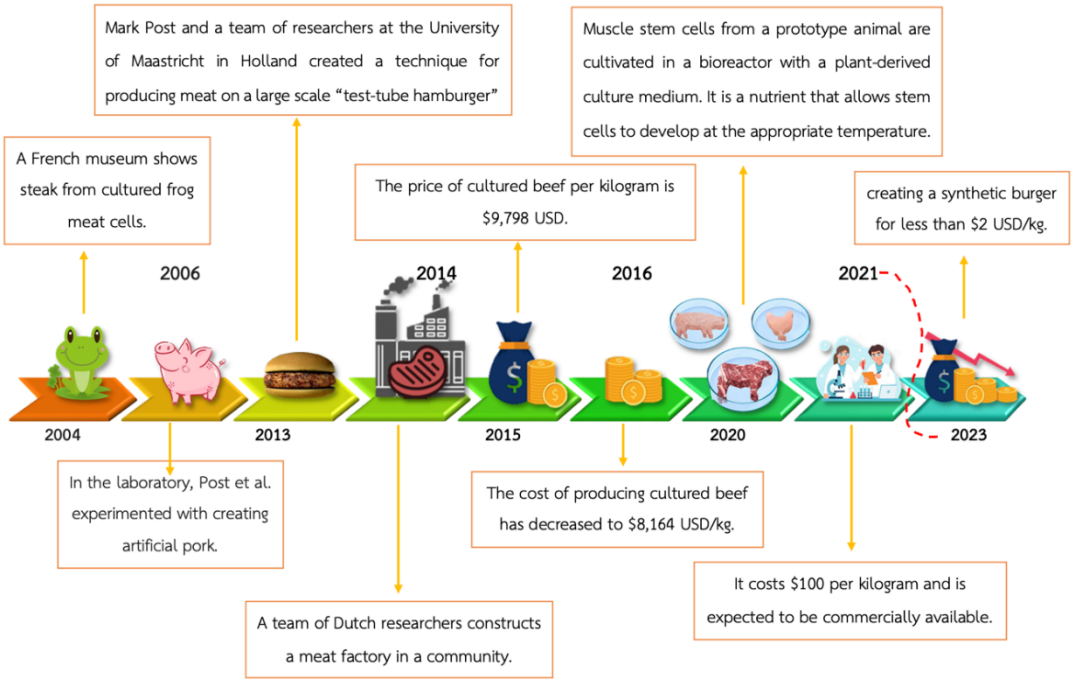

Figure 16 History of cultured meat

Source: Adapted from Aphirada (2014); Tummy (2019); H_2O (2020)

Meat eating is an essential component of a healthy diet. It is required by society and serves as a barometer of social development. The global expansion in meat production and consumption (FAO, 2014) has prompted environmental concerns regarding land and water demands, pollution, greenhouse gas emissions, and depletion (Steinfeld et al., 2006). Animal production contributes to biodiversity loss (Machvina et al., 2015), inefficient resource usage on ingested protein (Tilman et al., 2011), and greenhouse gas emissions from the consumption of meat (Gill et al., 2014). These represent concerns about food safety and human health risks, particularly zoonotic illnesses (Walker et al., 2005) and antibiotic use (Phillips et al., 2004). As well as the epidemiological link between red meat and processed meat consumption that causes disease in humans, such as colorectal cancer (Bouvard et al., 2015) or cardiovascular disease (Micha et al., 2010), animal welfare is becoming increasingly important to a pleasant environment and may also have a positive impact on health, although a low-meat diet is only beneficial to the environment

when grazing alongside streams, although people are frequently aware of these risks. (Macdiarmid et al., 2016). The high meat-eating trend persisted. This is frequently accomplished through coping mechanisms such as concealing the provenance of the meat. Strategic neglect or belief modification (Bastian and Loughnan, 2017; Onwezen and van der Weele, 2016) reduced antibiotic usage and more comprehensive health monitoring It improves resource usage by, for example, lowering the conversion rate of food to body weight. This leads to the quest for alternatives, such as plant-based and animal-based meat replacements, exotic sources (such as insects, cultured meat, and algae), and consumer-responsive strategies (Aiking et al., 2018). The pattern of high meat intake was maintained. This is frequently accomplished through coping mechanisms such as concealing the provenance of the meat. Strategic neglect or belief modification (Bastian and Loughnan, 2017; Onwezen and van der Weele, 2016) reduced antibiotic usage and more comprehensive health monitoring It improves resource usage by, for example, lowering the conversion rate of food to body weight. This leads to the quest for alternatives, such as plant-based and animal-based meat replacements, exotic sources (such as insects, cultured meat, and algae), and consumer-responsive strategies (Aiking, 2011).

Sustainable Profits Impact on Social Organizations' Market Power Economic survival and its implications for cultural and behavioral patterns of consumption in relation to alternative meat production are missing (Smetana et al., 2015; Spaargaren et al., 2013). The worth of alternative meat as a production system with technological, economic, environmental, social, and organizational implications. As a result, to compare alternative sustainability evaluations, a new conceptual framework, the Reflexive Integrative Comparative Heuristic (RICH), was proposed (desired dimensions), and changes in technological and social institutions required for beef substitutes were identified (the possibility dimension). This method helps us to answer the study topic of what the prerequisites and impact of meat replacement alternatives are in terms of meat quality and kind. This implies social and institutional transformation. This includes the proclivity to attain sustainability (van der Weele et al., 2019).

Cell culture medium

Cell culture medium is a basic substance used to create living cells. It is a recipe that has evolved over a lengthy period. There are two types of cell culture medium: natural and synthetic. Biological substances such as plasma, serum, or embryo extract are found in natural cell culture media. Synthetic cell culture medium, which consists of basal medium, can be supplemented with serum growth factor or hormones, whereas serum medium contains 5–10% serum and can be used with many cell lines. However, the makeup is frequently unknown. There are differences between manufacturing sites and production lots; it is costly and scaling up may be challenging. Medium without serum contains a well-known component. There is also a distinction between low-output batches. It makes scaling up your culture simpler. However, it is only effective for select cell lines. Some trace elements, such as Se, Fe, Cu, and Zn, are required in trace quantities. However, it is required, for example, as a co-factor in an enzyme-dependent process. A little variation in concentration can impair cell line generation efficiency, and trace elements including Ca, Na, Mg, and K are impurities in cell culture media. as well as other additions added to well-known nutrients such as bicarbonate, glutamine, HT, MTX, and MSX. The amount to apply is determined by the kind of cell. Typically, 2 g/l bicarbonate and 4–8 mmol/l glutamine are used.

The following are the key components of cell culture medium:

1. Cell growth nutrients, such as amino acids, vitamins, minerals, carbohydrates, and other components that the cell uses as an energy source for nutrients with a short half-life, such as glutamine amino acids, 4-6 weeks may necessitate the addition of more cell culture media. If food must be preserved for an extended period, a kind with a longer shelf life, such as L-alanyl glutamine, may be used.

2. Growth factors and hormones that are needed for controlling the rate of growth and other cell activities are often acquired by adding 5–20% of animal serum, such as bovine serum, which is also an excellent source of hormones. There are further properties such as proteins that aid in adhesion, proteolytic enzyme inhibitors, and so on. However, animal serum should be

used with caution due to the possibility of unstable quality. and might be infected with viruses or mycoplasma.

3. pH buffers for nutrients that maintain osmolality to avoid cell rupture (Kanokwan et al., 2019). (**Table 5**)

Table 5 Components of chicken cell culture medium

Substrates	Basal media	Serums	Others	Antibi-otics	Signaling molecules	Incubator conditions	Reference
Type I collagen and fibronectin	MEM for chicken	10% FBS+5% chicken serum			TGF-β	37 °C, 5% CO_2	Massague et al. (1986)
Collagen	MEM	15% HS	CEE			36.5 ˚C, 5% CO_2	Coleman and Coleman (1968)
	MEM	10% HS	CEE	P + S + F		37.5 °C, 5% CO_2	Trotter and Nameroff (1976)
Gelatin	MEM	10% HS	CEE	F + G		37.5 °C, 5% CO_2	Yablonka-Reuveni and Nameroff (1987)

Abbreviations: CEE, chick embryo extract; F, fungizone; FBS, fetal bovine serum; G, gentamycin; HS, horse serum; P + S, penicillin, and streptomycin; TGF- β, transforming growth factor beta; MEM, Eagle's media.

Laboratory meat production characterization

In vitro-cultured meat from animal cells provides the possibility for ethical management now. Environmental and public health concerns with meat production are extensively documented. as well as solving technological obstacles in the creation of cultured meat. Manufacturers and supporters of technology must consider societal concerns. Customer acceptability and attractiveness are included. Coverage in the media religious status, legislation, and possible economic consequences While much has been written on the attraction of customers and the

acceptability of cultured meat, less attention has been paid to how other parts of social media interact with this new technology. Broad social It has been proposed that moving to cultured beef offers enormous potential benefits. and has been carefully investigated for this fledgling business to realize its full potential (Bryant, 2020). Even though there are several technological issues related to the production of synthetic meat, by effectively developing this technology, at least some global problems can be overcome. The distinctions between conventional and laboratory animal husbandry were collected in this study. To acquire meat, the entire manufacturing system must be safe and environmentally responsible. Furthermore, there is a production technique that employs carbon dioxide in the creation process of synthetic meat. This is seen as a low-carbon livestock production strategy and another approach to reduce pollution. It will highlight present animal husbandry difficulties and create aquaculture meat production technologies to overcome them. particularly in three areas: 1) social and economic elements of cultured meat, 2) biological foundations of diverse meat cultures, and 3) technical methods of cultured meat production, etc. (**Table 6**)

A metabolomics study is also being conducted (the study of the diversity of cellular chemicals in relation to pathways and mechanisms in energy metabolism). Metabolism, in which the researchers used high-performance computers to mimic the metabolism system (to forecast the function and behavior of cells under environmental circumstances and over time) to enhance and analyze food quality. The skeletal muscle of farm animals and meat is one of the primary goals of metabolomics for meat characterization and biomarker research in production systems. Identifying possible meat control biomarkers (Muroya et al., 2014; Ma et al., 2017; Yu et al., 2019). By performing metabolomics analyses of meat quality, muscle, and meat in conjunction with meat quality characterization, with an emphasis on specific aspects connected to the animal's genetic background, sensory scores, or feeding and feeding systems. Meat may be preserved by methods like autopsy, processing, and sanitary management. Separation methods are used in most contemporary metabolomics studies.

Table 6 Comparison of traditional and cultured meat

Attributes	Traditional meat	Cultured meat	References
Production system			
Production method	Animal farming	Cell cultivation	Bhat et al. (2019)
Land requirement	4 m^2/bird/1.2 kg.	> 1 m^2/1.2 kg.	
Location of production	Mostly rural	Rural & urban	Bhat et al. (2019)
Production cost (So far)	80 bath	66 bath	
Production time	4 month	> 1 month	
Production yield (/100 m^2)	30 kg.	120 kg.	
Greenhouse gas emission	Very high	Low	Bhat and Fayaz (2011)
Energy requirement	4.5 GJ/t	18–25 GJ/t	Macleod et al. (2013);
Water and soil pollution	High	Low	Welin and Van der Weele (2012)
Sustainability	Low	High	Siegrist and Hartmann (2020)
Characteristics			
Manipulating composition	Impossible	Possible	Bhat and Fayaz (2011)
Human health	Low	High	Joshi et al. (2020)
Food safety	Low	High	Joshi et al. (2020)
Animal welfare	Low	High	Mouat and Prince (2018)
Ethical advantage	Low	High	Mancini and Antonioli (2020)
Consumer acceptance	High	Low	Siegrist et al. (2018)

Source: Hong et al. (2021)

Depending on the polarity and/or hydrophobicity of the target substance, electrophoresis (gas or liquid chromatography and capillary electrophoresis), mass spectrometry (MS), or nuclear magnetic resonance (NMR) procedures with multivariate analysis were used. These studies give helpful information for efficiently investigating meat quality aspects. Learn about the genetic basis and animal production practices that contribute to meat quality (Muroya et al., 2020). MS without pretreatment for biological samples under normal atmospheric conditions since it is based on atmospheric ionization Trends in the utilization of each

MEATabolomic technology for sample analysis, including CE-MS, GC-MS, LC-MS, NMR, and REIMS, have been investigated, and numerous sample analysis techniques have been included in certain cases. must be appropriate for each type of sample, as shown in **Table 7**.

Table 7 Overview of chicken metabolomics studies cited in this review.

Category of Objective	Factors Analyzed	Methodology	Multivariate Data Analysis	Ref. Authors
Meat abnormality	Dystrophy of breast	HR–MAS H–NMR	PCA, OPLS–DA	Sundekilde et al. (2017)
	GC–MS, LC–MS/MS	RF	H–NMR OPLS–DA	Abasht et al. (2017)
	Wooden breast	1H–NMR	OPLS–DA	Wang et al. (2020)
	Wooden breast	1H–NMR	OPLS–DA	Xing et al. (2020
	White striping	GC–MS, LC–MS	PCA, Pathway	Boerboom et al. (2018)
Processing, Spoilage	Marinade type, storage time, microbial load, sensory score	GC–MS	PCA, FDA	Lytou et al. (2018)
	Marinade type, marination time and temperature	LC	PCA	Lytou et al. (2017)
Authentication	Live/dead on arrival	LC–MS	PCA	Sidwick et al. (2017)
	Live/dead on arrival	LC–MS	PCA, Pathway	Cao et al. (2020)

FDA: factorial discriminant analysis; OPLS–DA: orthogonal PLS–discrimination analysis; Pathway: pathway enrichment analysis; PCA: principal component analysis; PLS: partial least square analysis; PLS–DA: PLS–discrimination analysis; Evaporative Ionization Mass Spectrometry; RF: random forest.

CHAPTER 3 MATERIALS AND METHODS

The development of cultured meat from embryonic stem cells of black-boned chickens and embryo fertility were initially studied, and embryonic stem cells were cultured under different conditions and made into cultured meat, as well as the protein qualification test of laboratory cultured black-boned chicken meat compared to naturally raised chicken meat. The research was divided into three experiments, as follows:

Experiment 1 Embryonic stem cell embryology studies

Stem cell

Chemical for embryonic stem cell culture

Chemical	Chemical formula	Lot number	Company
Cell culture			
Iscove's Modified Dulbecco's Medium (IMDM)		2141691	Gibco
Fetal bovine serum		42Q1670K	Gibco
Chicken serum			
Disodium hydrogen phosphate	Na_2HPO_4	F2066186918	Merck
Ethylenediaminetetraacetic acid	$C_{10}H_{16}N_2O_8$	BCCF3872	Sigma
Potassium chloride	KCl	K53031736121	Sigma
Potassium dihydrogenphosphate	KH_2PO_4	AM1206773017	Sigma
Sodium chloride	NaCl	K53031736121	Sigma
Sodium hydrogen carbonate	$NaHCO_3$	K53304229135	Sigma
Tris-hydrochloride	$NH_2C(CH_2OH)_3 \cdot HCl$	BCBX7213	Sigma
Antibiotic		2321086	Gibco
Trypsin 0.25%	$C_{39}H_{55}N_9O$	1906755	Gibco
Washing and stained cells			
PBS		2598B178	Amresco
PrestoBlue reagent		13846223A	Invitrogen
Proteinase K		NBE575875-F1	Carl roth

Fertilized eggs of Black-boned chickens were obtained from the Poultry farm, Faculty of Animal Science and Technology, Maejo university and incubated at 38.0 ℃ with 60% relative humidity (18 hours).

Isolation of black-boned chicken ES cells

Isolation of chicken ES cells were carried out following previously described method (Wu et al., 2010). In brief, blastoderm cells (stage X) were collected by syringe method in tissue culture dishes and rinsed with phosphate- buffered saline (PBS) to remove the yolks by centrifugation at 1,000 rpm for 10 min and suspended. ES cells were maintained in a 5% CO_2 humidified atmosphere at 37.0℃ with 5 ml. Iscov's Modified Dulbecco's Medium (IMDM, Gibco, Grand Island, N. Y., USA) and 10% FBS. Count the number of cells in a hemocytometer (N= $2x10^4$ cells) for culture.

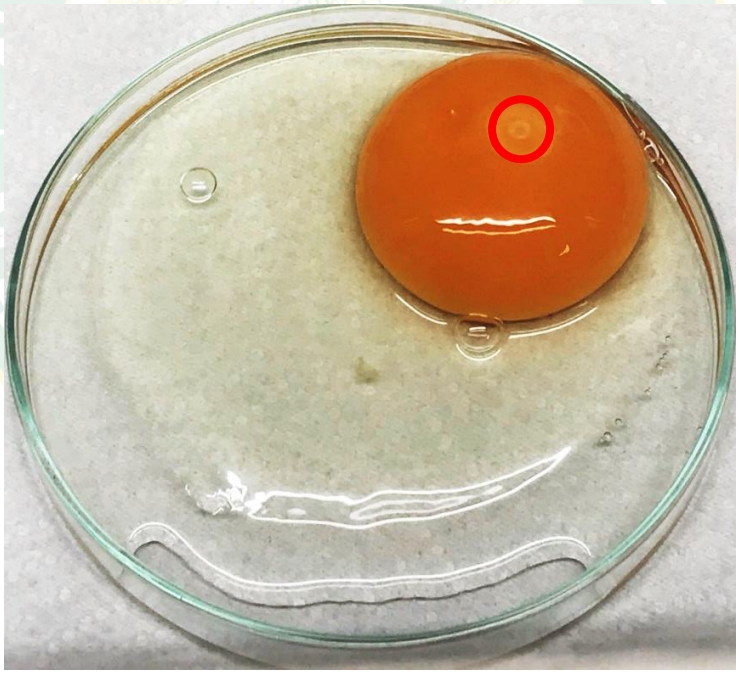

Figure 17 Location of the collected stem cells

The measurement of black-boned chicken ES cells growth

The cultivated stem cells were dripped with 0.05% Trypsin, put in a cell culture flask, the media was withdrawn, and 100 ul of fresh medium was added to 10 ul/well of 12 mM MTT (Component A) and incubated at 37 ℃ for 4 hours before

adding 100 ul/well of SDS (Component B). In a humidity chamber, mix the microplate and incubate it for 4 hours at 37 °C. Pipette the sample and measure the absorbance at 450 nm. (UVmini-1240, Shimadzu, Europe) and used a hemocytometer to count the amount of black-boned chicken ES cells.

Experiment 2 Culture of embryonic stem cells and meat in various media ingredients

Cultured meat

Chemical for cultured meat

Chemical	Chemical formula	Lot number	Company
Cell culture			
Iscove's Modified Dulbecco's Medium (IMDM)		2141691	Gibco
Fetal bovine serum		42Q1670K	Gibco
Chicken serum (comercial)			
Black-Boned chicken serum			
Pradu Hang Dum chicken serum			
Antibiotic		2321086	Gibco
Washing and stained cells			
PrestoBlue reagent		13846223A	Invitrogen
Trypan blue	$C_{34}H_{28}N_6O_{14}S_4$	2101677	Gibco
Trypsin 0.50%	$C_{39}H_{55}N_9O$	1917558	Gibco

Cultivation of black-boned chicken ES cells

The quantity of cells is 2-4 x10^4 cells/ml, as stated in the section on stem cell sorting. ES cells were maintained in a 5% CO_2 humidified atmosphere at 37.0 °C with 5 ml. Iscov's Modified Dulbecco's Medium (IMDM, Gibco, Grand Island, N. Y., USA) and 10% serum (Fetal Bovine; FBS (T1), Commercial chicken; SCK (T2), Pradu Hang Dam chicken (T3), Black-Boned chicken, BBC (T4)). The cell colonies were digested at 37.0

°C for 2 to 3 min with 0.25% trypsin. The dissociated ES clusters were suspended with pipete and sub-cultured in a 5% CO_2 humidified atmosphere at 37.0°C with culture flasks containing feeder cell layer and IMEM medium. The medium was replaced 3 days with half fresh medium by trypsin 0.25% (w/v) (Every 12 hr., keep an eye on one of the cells) Exclusion of Trypan blue as a vitality dye. To segregate cell properties, ELISA was used to assess the existence of ESC and Store at -80 °C.

Black-boned chicken cultured meat cultivation

Following stem cell culture, remove the freezing media by centrifugation at 1,500 rpm for 10 min, suspended, and media added. Cultured meats were maintained in a 5% CO_2 humidified atmosphere at 37.0°C with Iscov's Modified Dulbecco's Medium (IMDM, Gibco, Grand Island, N. Y., USA) and 10% serum (Fetal Bovine; FBS (T1), Commercial chicken; SCK (T2), Pradu Hang Dam chicken (T3), Black-Boned chicken, BBC (T4)). The cell colonies were digested at 37.0 °C for 2 to 3 min with 0.50% trypsin. The dissociated ES clusters were suspended with pipette and sub-cultured in a 5% CO_2 humidified atmosphere at 37.0°C with culture flasks containing feeder cell layer and IMEM medium. The medium was replaced 3 days with half fresh medium.

The measurement of black-boned chicken ES cells growth

The cultivated stem cells were dripped with 0.05% Trypsin, put in a cell culture flask, the media was withdrawn, and 100 ul of fresh medium was added to 10 ul/well of 12 mM MTT (Component A) and incubated at 37 °C for 4 hours before adding 100 ul/well of SDS (Component B). In a humidity chamber, mix the microplate and incubate it for 4 hours at 37 °C. Pipette the sample and measure the absorbance at 450 nm. (UVmini-1240, Shimadzu, Europe) and used a hemocytometer to count the amount of black-boned chicken ES cells.

Statistical analysis

All data were performed by using the Completely randomized design (CRD) in SPSS software for significant difference ($P < 0.05$) and the results were presented as means ±standard error (SD) (Steel et al., 1997)

Experiment 3 Compared the protein content of laboratory-cultured black-bone chicken meat to wild-farmed black-bone chicken meat

The study gathered four techniques for comparing the protein characteristics of laboratory-cultured black-bone chicken meat to naturally raised chicken meat, as follows:

1. Nutritional analysis of chicken meat by Proximate analysis (AOAC, 2000)
2. Protein analysis by nitrogen combustion method
3. Observe the characteristics of the cultured meat by section meat method
4. Examining muscle fiber appearance by Scanning Electron Microscopy; SEM
5. The areas of fibers were identified used Digital Microscope / Camera Software Motic® Images Plus (v. 2.0)

Statistical analysis

All data were performed by using the Completely randomized design (CRD) in SPSS software for significant difference ($P < 0.05$) and the results were presented as means ±standard error (SD) (Steel et al., 1997)

CHAPTER 4 RESULTS AND DISCUSSION

Experiment 1 Embryonic stem cell embryology studies

At 48 hours, ES cells start dividing again. As shown in **Figure 18**, it has a fixed number of cells, indicating that cell division has ended. Whether it will continue to grow is dependent on a variety of parameters, such as medium, serum, and others. This is according to the cell growth or cell cycle (Cell division) theory, which corresponds to the research of Chapman et al. (2001), used the EC culture method and confocal microscopy to visualize gastrulation movements as they occurred, electroporated stage X chick embryos with a Green Fluorescent Protein (GFP) reporter gene, and followed the behavior of electroporated cells at stage 3 . As gastrulation movements occurred, most cells rapidly separated from each other in places distant from the primitive streak. This finding contrasts sharply with previous findings in other epithelia (e.g., Xenopus embryos, C. elegans, Drosophila, and zebrafish), where cells almost always remain in contact (Gibson et al., 2006 ; Kieserman et al., 2008; Bischoff and Cseresnyés, 2009; Harrell and Goldstein, 2011; Campinho et al., 2013). G1 phase and G2 phase are two phases in the interphase of the cell cycle. The duration of the cell cycles varies according to the type of organisms. G1 phase is the first substage of interphase. G2 phase is the final substage of interphase. Significant development processes occur within the cell at G1 phase. When compared with G1 phase G2 phase is a shorter phase. Proteins that synthesize during G1 phase include mainly histone proteins and most RNA synthesized is mRNA. If a cell enters the G2 phase, it confirms the fact that the cell has completed the S phase where DNA replication has taken place. Cell cycle regulatory mechanisms will control both phases. (Alberts, 2017; Cooper et al., 2007). The cell cycle begins in interphase with cell preparation before division, followed by cell proliferation in the M phase. As a result, cell development requires the production of enzymes and proteins required for DNA synthesis to 1) begin the cell cycle again (G0) or 2) differentiate to become a specialized cell (G1 phase). G1 cyclin-dependent kinase-cyclin complexes control and stimulate cells into the following S phase via CdkC

(G1CdkC). S-phase (DNA synthesis) is the sole phase of DNA synthesis in which CdkC exclusively regulates DNA proliferation. The number of times chromosomes were created at this stage rose from one to one. The G2 phase is responsible for the production of extra proteins and RNA required for continued cell division. The G1, S, and G2 phases are referred to as interphase (Suwansan, 2017; PaphatPremsiri, 2017). When stored at -80 °C, it may still increase the number of cells. As a result, the cells cultured in this research are embryonic cells originating from black-bone chicken eggs. The researcher started with 10% FBS as a medium and feeder cell. Most researchers use FBS as a media component.

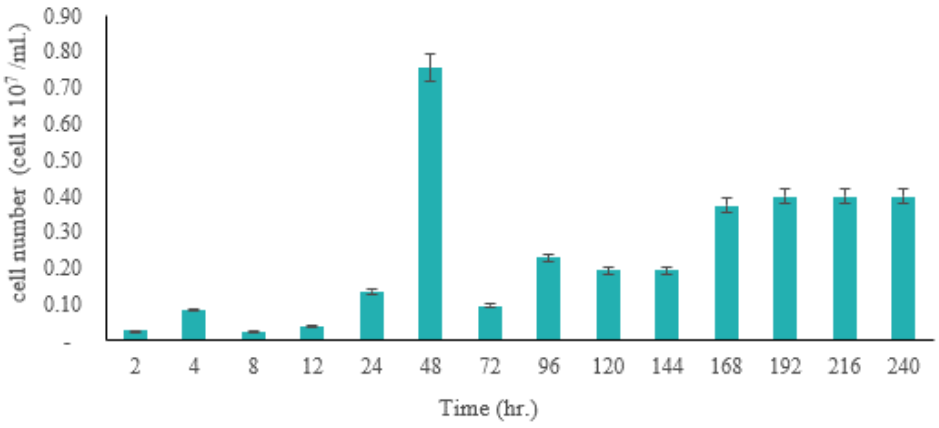

Figure 18 The cell number of black-boned chicken ES cells in 10%FBS medium

Experiment 2 Culture of embryonic stem cells and meat in various media ingredients

The absorbance of black-boned chicken ES cells was determined at 450 nm. Found that, T4 grew faster than the other groups after cultivated stem cells two hours. T3 grows faster than any other group between 4 and 12 hours. T4 has grown more than any other group in 24 hours. T2 had more growth than the other groups at 48 - 192 hours, while T4 has greater growth between 216 and 240 hours. ($P<0.05$) shown in **Table 8**. This follows the same cell growth or cell cycle (cell division) theory as experiment 1. Counting ES cells of end process, T4 had a significantly higher number and growth rate than the other groups ($P < 0.05$) shown in **Figure 19**.

Table 8 Growth of black-boned chicken ES cells at absorbance of OD 450 nm.

Treatment	Time (Hours)													
	2	4	8	12	24	48	72	96	120	144	168	192	216	240
T1	0.019^{d}	0.017^{d}	0.020^{d}	0.019^{d}	0.016^{d}	0.027^{d}	0.007^{d}	0.011^{d}	0.010^{c}	0.033^{c}	0.078^{b}	0.055^{c}	0.017^{d}	0.017^{d}
T2	0.039^{b}	0.030^{c}	0.021^{c}	0.034^{c}	0.052^{b}	0.129^{a}	0.163^{a}	0.326^{a}	0.122^{a}	0.353^{a}	1.027^{a}	1.159^{a}	0.030^{c}	0.030^{c}
T3	0.037^{c}	0.048^{a}	0.052^{a}	0.046^{a}	0.043^{c}	0.045^{c}	0.025^{c}	0.012^{c}	0.052^{b}	0.029^{d}	0.047^{c}	0.035^{d}	0.098^{b}	0.098^{b}
T4	0.054^{a}	0.038^{b}	0.044^{b}	0.035^{b}	0.100^{a}	0.104^{b}	0.059^{b}	0.059^{b}	0.001^{d}	0.074^{b}	0.045^{c}	0.058^{b}	0.179^{a}	0.179^{a}
SEM	0.004	0.003	0.004	0.003	0.009	0.012	0.018	0.039	0.014	0.040	0.126	0.144	0.019	0.019
P-value	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001

T1: Fetal Bovine, FBS; T2: Commercial chicken, SCK; T3: Pradu Hang Dam chicken, PDC; T4: Black-Boned chicken, BBC

$^{a-d}$ values in columns with different letters differ significantly (P<0.05)**;** SEM mean Standard error mean

Most chicken embryonic stem cells are isolated from stage X blastoderm cells in preparation for embryonic stem cell cultivation with a variety of feeder cells. Growth factors and cytokines are also present (Aubel and Pain, 2013). Similarly, mouse embryonic stem cells (mESC) may maintain embryonic stem cells. Current embryonic stem cell isolation methods isolate pluripotent embryonic cells from undifferentiated pluripotent embryonic stages using leukemia inhibitory factor (LIF) as a feeder cell (Horiuchi et al., 2006). In vitro, both primordial germ cells (PGCs) and spermatogonial stem cells (SSCs) may develop into neuroblast-like adipocytes and osteoblasts and display gene markers. comparable (Li et al., 2010; Jung et al., 2007). Consequently, using chicken black-bone serum as a feeder cell in a cell culture medium is a feasible alternative for clearly observing growth results.

Thus, chicken embryonic stem cells are derived from embryos and cultivated in the laboratory. Which are collected from diverse sources at various stages of embryonic development and may test the fact that it is a pluripotent embryonic cell since it can form an embryo and split into a single cell, making it equivalent to mammalian stem cells. This serves as a model for research into stem cell biology, etc. Long-term continuous culture of stem cells in co-culture with peer cells was effective in terms of cell growth and preservation. Several types of steer cells have been used in poultry stem cell cultures, including Sandoz inbred mouse-derived Thioguanine resistant and Ouabain-resistant (STO), mouse fibroblast feeder (MEF), and chicken embryonic fibroblast (CEF) (Van De Lavoir et al., 2019; Wang et al., 2020). However, utilizing heterogeneous animal cells as feeder cells increases the chance of progenitor cells becoming contaminated with secretions from the heterogeneous feeder cells and the activation of those molecules may change the characteristics of stem cells. The use of the feeder cell type as the stem cell decreases contamination (Naito et al., 2015). The chicken embryonic stem cell culture medium was mixed with BBC in this study. Consequently, the embryo grows faster than with other serums.

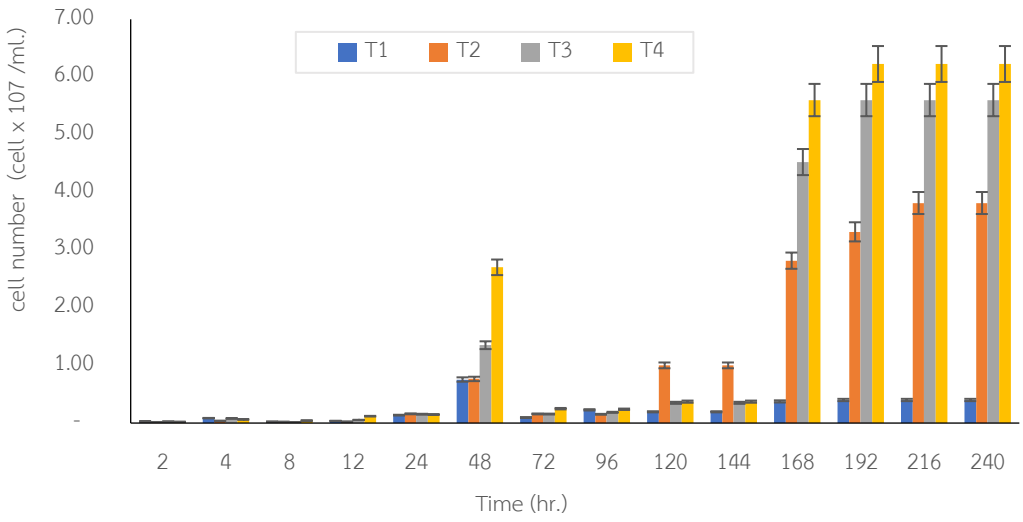

Figure 19 The cell number of black-boned chicken ES cells

Experiment 3 Compared the protein content of laboratory-cultured black-bone

The protein percentage of laboratory-cultured black-bone chicken meat is compared to that of wild-farmed chicken meat by using the combustion technique and proximate method of CP to determine whether the cultured chicken meat qualifies as chicken meat. Found that T4 had significantly more protein than the other groups ($P < 0.05$), followed by T2, T3, and T1, respectively. While T1 had significantly more EE and GE of meat than the other groups ($P < 0.05$), T3, T2, and T4 followed. Since cultured meat tends to be similar in protein to chicken meat and comparable to real chicken meat, as shown in **Table 9**. Since the cells were muscle cells, it is expected that their protein analysis by proximate analysis and the combustion method provided was greater than that of chicken thighs raised in the conventional manner.

Table 9 Protein analysis by the proximate analysis and combustion method produced (% of DM basis)

Item	AD	DM	DM basis			
			Combustion	CP	EE	GE (kcal/100 g)
T1	31.98[a]	90.84	72.81[d]	61.39[d]	37.15[a]	685.52[a]
T2	26.08[c]	90.66	91.88[b]	87.08[b]	11.38[c]	603.25[c]
T3	27.34[b]	91.77	84.16[c]	83.13[c]	17.91[b]	618.37[b]
T4	12.17[d]	89.94	99.25[a]	97.71[a]	0.43[d]	491.44[d]
SEM	1.910	0.317	2.953	3.986	4.026	21.056
P-value	<0.001	0.252	<0.001	<0.001	<0.001	<0.001

* For analysis, chicken thigh meat was used.

DM: Dry matter basis; AD: Air dry basis; CP: Crude Protein; EE: Ether extract; GE: Gross energy

T1: Commercial chicken meat; T2: Pradu Hang Dam chicken meat; T3: Black-Boned chicken meat; T4: Culture meat

[a-d] values in columns with different letters differ significantly ($P<0.05$); SEM mean Standard error mean

When we observe the appearance of muscle fiber under a scanning microscope (A: 2,000x; B: 10,000x) and compound microscope (C: 200x), we can observe that the characteristics of the cultured meat are not different from normal chicken cells, as shown in **Figure 20**. The muscles cell of SCK were arranged in bundles separated with loose perimysium. The muscle cells were large spindle shaped, periphery placed nuclei, dense muscular fiber. Other supportive tissues, such as adipose tissue and vascular structures were demonstrated and comprise 10-20% of the submitted samples. PDC muscles cell were arranged in small bundles separated with thin perimysium. The muscle cells were large spindle shaped, periphery placed nuclei, dense muscular fiber. Few adipose tissues (~5 %) were observed within the tissues. The muscles cell of BBC was arranged in large bundles and fascicles separated with thin perimysium. The muscle cells were large spindle shaped, periphery placed nuclei, dense muscular fiber. Multifocal pigmentations were noted in the connective tissue and collagenous fibers. Surrounded adipose tissues, approximately 10-15%, were include within the muscular structures. CTM cell were arranged in bundles, fascicles, reticulated, and occasionally separated individual cells. The muscle cells were spindle shaped, periphery placed nuclei, dense muscular fiber. Variably sized of the myocytes were noted. Similar to Zhu et al. (2021), characterization of muscle development and gene expression in early embryos of chicken, quail, and their hybrids were studied, characterization of muscle it same this study.

After measuring the cross-sectional size of the muscle cells under the microscope, it was found that the median cross section area (4,214.49 μm^2) and diameter (99.43 μm.) of the SCK muscle were significantly higher ($P < 0.01$) than those of the other groups. But at the same time, the cross-sectional size of the culture meat cells (area: 2,635.33 μm^2 and diameter: 64.75 μm.) was not different from that of the two types of native chicken muscle cells (area: 2,674.32 μm^2 and diameter: 65.23 μm. in PDC; area: 3,366.36 μm^2 and diameter: 73.18 μm. in BBC) ($P > 0.05$). On the other hand, the cross-section of the artificial culture chicken muscle cells made by this research is the same as that of normal chicken muscle cells. The

same could possibly be said about Sartell (2 0 18) studies on chicken cell characterization for measurement of muscle fiber size using the Imaris software. Similar to Kui et al. (2 0 2 2), gene expression profiles of specific chicken skeletal muscles were studied, the median diameter (71.41 μm) and CSA (4,136.00 μm^2) of the quadratus lumbrorum muscle were identified using Digital Microscope / Camera Software Motic® Images Plus (v. 2.0). (**Figure 21-22**)

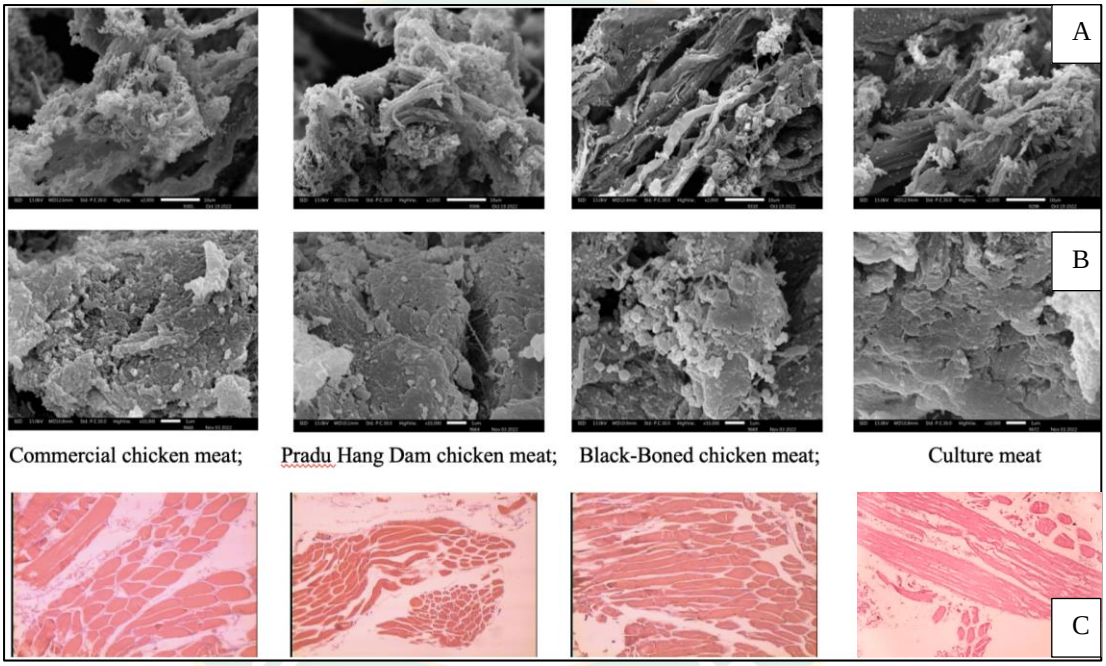

Figure 20 Characterization of muscle fiber under scanning microscope (A:2,000x; B: 10,000x) and compound microscope (C: 100x)

Source: The lab test at The Veterinary Diagnostic Center (VDC), Chiang Mai University Animal Hospital, Chiang Mai University

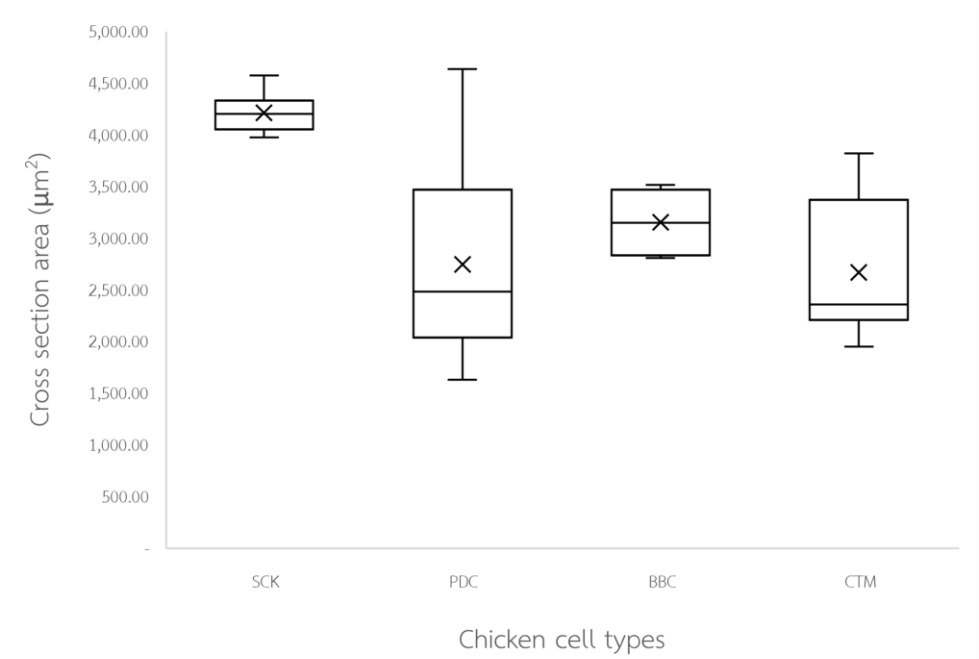

Figure 21 The cross-sectional area of the muscle cells measured under the microscope

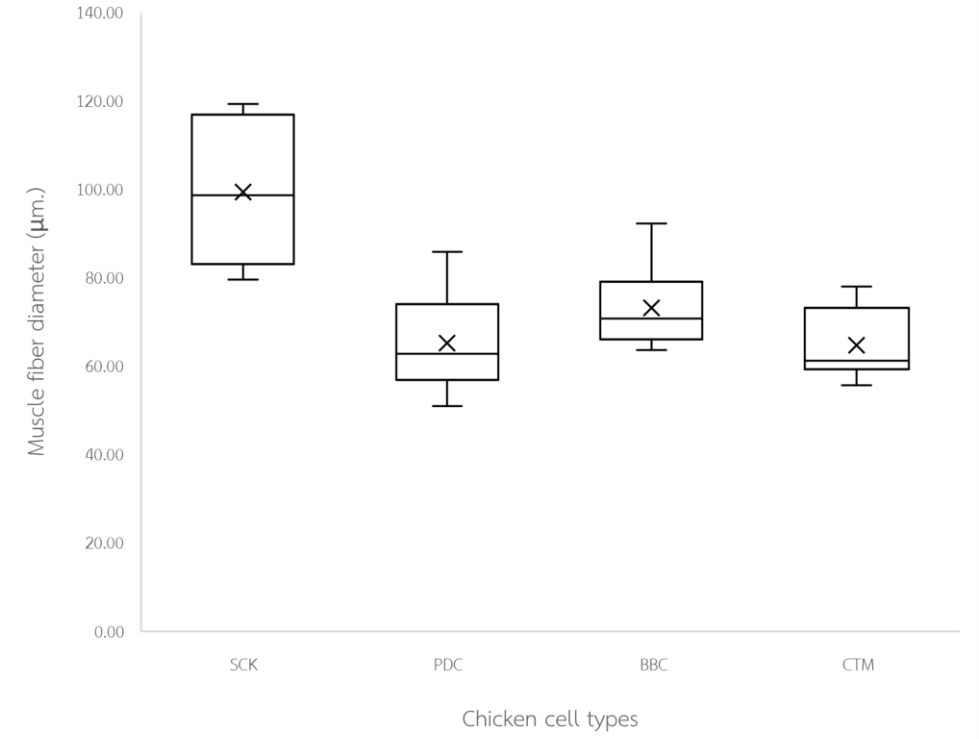

Figure 22 The muscle fiber diameter of the muscle cells cross-sectional measured under the microscope

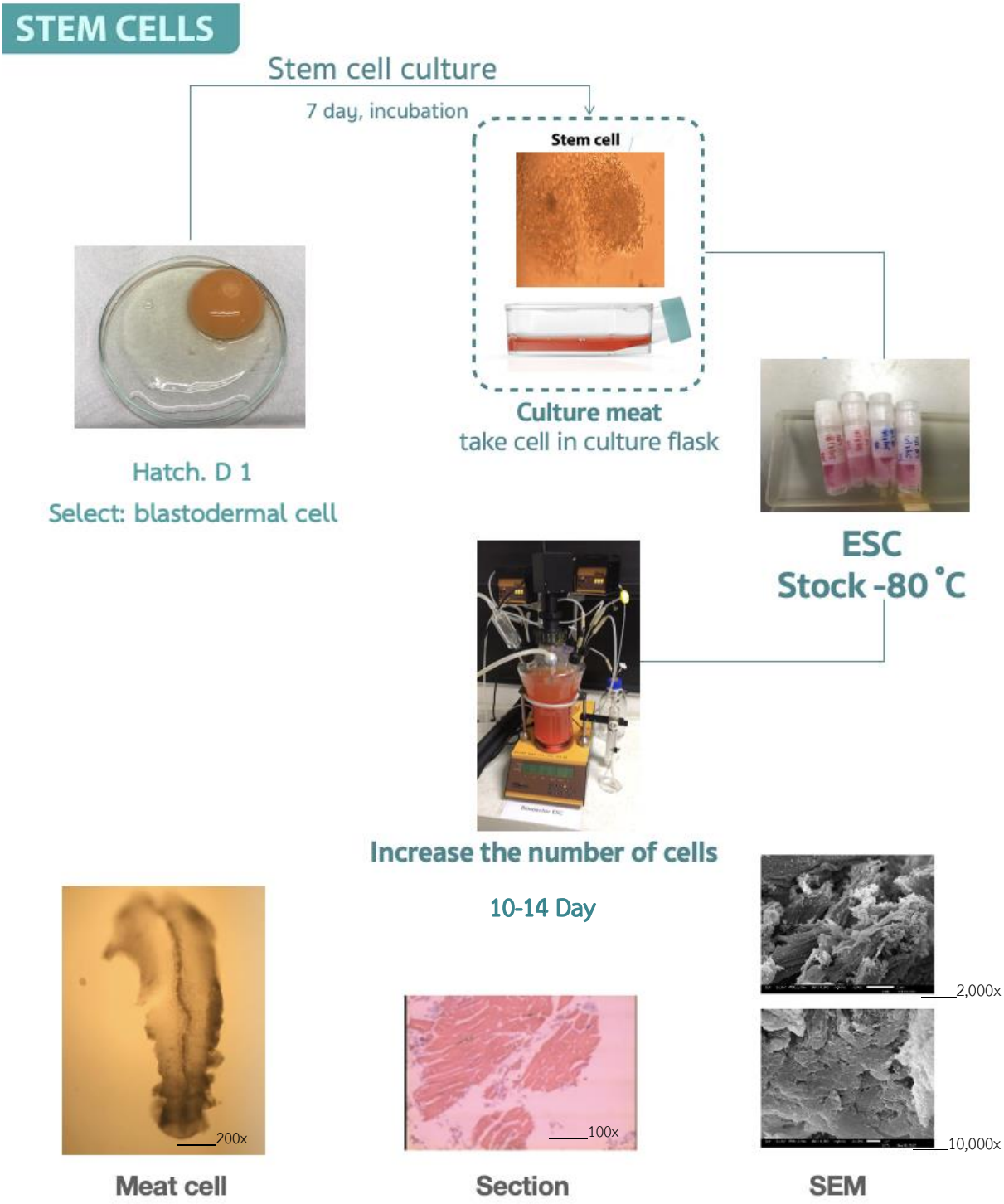

Figure 23 Process of develop cultured meat from black-boned chicken embryonic stem cell

CHAPTER 5 CONCLUSION AND SUGGESTION

CONCLUSION: The fact that cultured meat can be developed from black-boned chicken embryonic stem cells in part based on their growth performance emphasizes the fact that the cultivation of black-boned chicken embryonic stem cells for cultured meat necessitates the use of a medium containing black-boned chicken serum. Furthermore, based on the findings of the protein analysis by combustion method, cultured meat is comparable to real chicken meat and characteristics of the cultured meat are not different from normal chicken cells. It also has the same color as black-boned chicken meat due to the presence of melanin in cultured meat.

SUGGESTION: The meat culture lab should be clearly separated from other rooms, and cleanliness and ease of use should be top priorities to keep the cultured meat as clean as possible and prevent contamination as much as possible, and development of cultured meat from other poultry embryonic stem cells.

FUTURE RESEARCH: Increase the total number of cells in an industrial-scale bioreactor and in-depth characterization of muscle cells derived from cultured meat.

APPENDIXES

Dr. Wiboon Lapchatuporn Fund, Intech Feed Co., Ltd. offered a dissertation scholarship from the The Animal Husbandry Association of Thailand Under the Royal Patronage of H.R.H. Princess Maha Chakri Sirindhorn

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- Research assistant in the project improvement of cattle production system in Member of the Sisaket Farmers Council with reproductive biotechnology and animal feed technology to increase the pregnancy rate of cows.

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2018 – 2019 - Research assistant in the project to enhance economic returns of beef cattle supply chains in the Northern Region

- Research assistant in the project improvement of the beef cattle production system in Lampang Province with reproductive biotechnology and feed technology knowledge to increase the pregnancy rate of cows.

2017 - 2018 Research assistant in the project the use of phenolic extract from Longan seed supplemented in the formulation of swine sausage products to replace synthetic supplements for extend the shelf life and maintain the quality of the products.

(Work scope: extract of phenolic from Longan seed, make sausage products, and meat quality laboratory)

2016 - 2018 Research assistant in the project effects of dietary Lys and Met levels on growth performance, carcass composition, and meat quality in Black-boned chickens at 0-12 weeks of age, and Effects of Free-Range Rearing System on Carcass Composition and Meat Quality of Black-boned chickens and Broiler.

(Work scope: Mixed feed, raised the Black-boned chickens, save and store data for calculated growth performance (feed intake, body weight gain, and feed conversion ratio) of Black-boned chickens at 0-4, 4-8, and 8-12 weeks of age. After that, save and store data for calculated carcass composition, and meat quality of Black-boned chickens at 4, 8, and 12 weeks of age and 35 days of broiler)

2015 – 2016 Academician at Sun Food International Co., Ltd., SARABUREE, THAILAND.

(Work scope: Find solutions and suggestion in the chicken farm, such as 1.) The use of herbs to kill rats on the farm for they are not resistant from drugs, 2.) Early death resolution of chicks, 3.) Preparation of basic chicken health check-up programs, such as primary screening for external health and chicken's carcass. Then order the medication and ordered the farmer to clean the expected part that was expected of the disease, etc.)

PUBLICATIONS:

1. Yammeun-Art, S., P. Somrak, and C. Phatsara. 2017. Effect of the ratio of maize cob and husk to napier Pakchong 1 silage on nutritive value and in vitro gas production of rumen fluid of Thai native cattle. Animal Production Science, 57(8): 1603-1606.
2. Somrak, P., P. Tarachai, J. Panatuk, K. Chukiatsiri, and K. Chompupun. 2018. Effects of Dietary Lysine and Methionine Levels on Growth Performance, Carcass Compositions and Meat Quality in Black-Boned Chickens at 0-4 Weeks of Age. The 7th National Animal Science Conference of Thailand 2018. Journal of Agricultural Research and Extension 35(2) (Suppl. 2): 41-48.
3. Panatuk, J., P. Somrak, K. Hamprakorn, and P. Tarachair. 2017. Effect of Longan Seed and Tamarind Seed Extract Solution on Beef Quality under Refrigerated Storage. The 6th National Animal Science Conference of Thailand 2017. Agricultural Science Journal 2017 Vol. 48 (2) (Suppl.): 868-874.
4. Tarachai, P., J. Panatuk, K. Chompupun, K. Chukiatsiri, P. Somrak, R. Kanrut, P. Tippawong, and N. Saiyatha. 2018. Effects of Sex and Free-Range Rearing System on Carcass Composition and Meat Quality of Broiler. The 7th National Animal Science Conference of Thailand 2018. Journal of Agricultural Research and Extension 35(2) (Suppl. 2): 855-863.
5. Chompoopan, K., P. Tarachai, Y. Sunanta, S. Khumput, K. Chukiatsiri, P. Promtan, and W. Pattanawong. 2022. Developing the Fa Luang black bone chicken's species for high quality and hatching of farmer community in the farming system. The 10th National Animal Science Conference of Thailand 2022. Thai journal of animal science 2022 vol. 3 (Suppl. 1): 16-21.
6. Sunanta, Y., P. Tarachai, K. Chompoopan, S. Khumput, K. Chukiatsiri, P. Promtan, and W. Pattanawong. 2022. Development of an organic Fa Luang black bone chicken nutrient that is suitable for raising the Fa Luang black bone chicken of the farmers community. The 10th National Animal Science

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2. Participate in a workshop, "Workshop Design Thinking" (MAP, Maejo university)
3. Portrait photography classes online (Thai MOOC)
4. Participate in the project "Research to market: R2M Nos. 9 (Gen Kai for the future team) and 10 (Tai Tao team) " (MAP, Maejo university); University and regional level

REFERENCES

Abasht, B., M. F. Mutryn, R. D. Michalek, and W. R. Lee. 2016. Oxidative stress and metabolic perturbations in wooden breast disorder in chickens. **PloS one**, 11(4): 1-16.

Acloque, H., V. Risson, A. M. Birot, R. Kunita, B. Pain, and J. Samarut. 2001. Identification of a new gene family specifically expressed in chicken embryonic stem cells and early embryo. **Mechanisms of Development**, 103: 79-91.

Alberts, B. 2017. **Molecular biology of the cell**. WW Norton & Company.

Alexander, P., C. Brown, A. Arneth, C. Dias, J. Finnigan, D. Moran, and M. D. Rounsevell. 2017. Could consumption of insects, cultured meat or imitation meat reduce global agricultural land use?. **Global Food Security**, 15: 22-32.

AOAC. 2000. **Official methods of analysis of AOAC International**. AOAC International.

Bischoff, M., and Z. Cseresnyés. 2009. **Cell rearrangements**, cell divisions and cell death in a migrating epithelial sheet in the abdomen of Drosophila.

Boerboom, G., T. van Kempen, A. Navarro-Villa, and A. Pérez-Bonilla. 2018. Unraveling the cause of white striping in broilers using metabolomics. **Poultry Science**, 97(11): 3977-3986.

Bouahom, B., S. Keonouchanh, and S. Khamphavong. 2007. **Indigenous chickens: an important part of rural livelihoods in the Lao People's Democratic Republic**. In: People and Animals – Traditional Livestock Keepers: Guardians of Domestic Animal Diversity. Food and Agriculture Organization of the United Nations (FAO). Rome. 71–78.

Campinho, P., M. Behrndt, J. Ranft, T. Risler, N. Minc, and C. P. Heisenberg. 2013. Tension-oriented cell divisions limit anisotropic tissue tension in epithelial spreading during zebrafish epiboly. **Nature cell biology**, 15(12): 1405-1414.

Chaiwat, S. 2020. **2020-2022 Industry Business Trends: Chilled, Frozen, and Processed Chicken**. [Online] Available: https://www.krungsri.com/th/research/ industry/-industry-outlook/Food-Beverage/Frozen-Processed-Chicken/IO/io-frozen-processed-chicken.

Chapman, S. C., J. Collignon, G. C. Schoenwolf, and A. Lumsden. 2001. **Improved method for chick whole‐embryo culture using a filter paper carrier**. Developmental dynamics: an official publication of the American Association of Anatomists, 220(3): 284-289.

Coleman, J. R., and A. W. Coleman. 1968. Muscle differentiation and macromolecular synthesis. **Journal of Cellular Physiology**, 72(2): 19–34.

Cooper, G. M., R. E. Hausman, and R. E. Hausman. 2007. **The cell: a molecular approach**. Washington, DC: ASM press. Vol. 4: 649-656.

Del Favero, S., H. Roschel, M. Y. Solis, A. P. Hayashi, G. G. Artioli, M. C. Otaduy, and B. Gualano. 2012. Beta-alanine (Carnosyn™) supplementation in elderly subjects (60–80 years): effects on muscle carnosine content and physical capacity. **Amino acids**, 43(1): 49-56.

Dogru Abbasoglu, S., N. Koçak-Toker, and M. Uysal. 2018. Carnosine as a Putative Antioxidant in Usage Against Liver Disease. **Journal of Liver**. Academic Press: 295-304.

Dorshorst, B., R. Okimoto, and C. Ashwell. 2010. Genomic regions associated with dermal hyperpigmentation, polydactyly and other morphological traits in the Silkie chicken. **Journal of Heredity**, 101(3): 339-350.

Faibis, N. 2016. **How important is protein, how much do you eat to get enough?** [online] Available: Protein, how important it is, how much to eat to get enough - pobpad (pobpad.com). (20 August 2021)

FAO. 2021. **Meat and meat products. food outlook biannual report on global food markets, june 2021**. Food and Agriculture Organization of the United Nations (FAO). Rome. 176 p.

Gibson, M. C., A. B. Patel, R. Nagpal, and N. Perrimon. 2006. The emergence of geometric order in proliferating metazoan epithelia. **Nature**, 442(7106): 1038-1041.

H_2O. 2020. **Singapore's first nation to sell 'Meat from the lab' food of the future that may be closer than you think.** Thairath online [online] Available: https://www.-thairath.-co.th/news/foreign/1988705. (28 July 2021).

Habeeb, A. A., and A. A. EL-Tarabany. 2018. Impact of environmental pollution on healthy

and productivity of farm animals.**American international journal of multidisciplinary scientific research**, 1(3):17-25.

Harrell, J. R., and B. Goldstein. 2011. Internalization of multiple cells during C. elegans gastrulation depends on common cytoskeletal mechanisms but different cell polarity and cell fate regulators. **Developmental biology**, 350(1): 1-12.

Jung, J. G., D. K. Kim, T. S. Park, S. D. Lee, J. M. Lim, and J. Y. Han. 2005. Development of novel markers for the characterization of chicken primordial germ cells. **Stem Cells**, 23(5): 689-698.

Jung, J. G., Y. M. Lee, T. S. Park, S. H. Park, J. M. Lim, and J. Y. Han. 2007. Identification, culture, and characterization of germline stem cell-like cells in chicken testes. **Biology of reproduction**, 76(1): 173-182.

Khumpeerawat, P., M. Duangjinda, and Y. Phasuk. 2021. Carnosine Content and Its Association with Carnosine-Related Gene Expression in Breast Meat of Thai Native and Black-Bone Chicken. **Animals**, 11(7): 1987.

Kieserman, E. K., M. Glotzer, and J. B. Wallingford. 2008. Developmental regulation of central spindle assembly and cytokinesis during vertebrate embryogenesis. **Current Biology**, 18(2): 116-123.

Kui, H., B. Ran, M. Yang, X. Shi, Y. Luo, Y. Wang, and M. Li. 2022. Gene expression profiles of specific chicken skeletal muscles. **Scientific Data**, 9(1): 552.

Lavial, F., and Pain, B. 2010. Chicken embryonic stem cells as a non-mammalian embryonic stem cell model. **Development, growth, and differentiation**, 52(1): 101-114.

Lavial, F., G. Montillet, E. Bachelard, J. Samarut, and B. Pain. 2006. Chicken stem cells as a model to generate transgenic chicken: present and perspectives. **Journal of Poultry Science**, 43(4): 313-322.

Li, L., H. H. Liu, F. Xu, J. M. Si, J. Jia, and J. W. Wang. 2010. MyoD expression profile and developmental differences of leg and breast muscle in Peking duck (Anas platyrhynchos Domestica) during embryonic to neonatal stages. **Micron**, 41(7): 847-852.

Li, Y. F., R. R. He, B. Tsoi, and H. Kurihara. 2012. Bioactivities of chicken essence. **Journal of Food Science**, 77 (1): 105–110.

Ma, D., Y. H. B. Kim, B. Cooper, J. H. Oh, H. Chun, J. H. Choe, and B. Min. 2017. Metabolomics profiling to determine the effect of postmortem aging on color and lipid oxidative stabilities of different bovine muscles. **Journal of agricultural and food chemistry**, 65(31): 6708-6716.

McClements, D. J., and L. Grossmann. 2021. The science of plant-based foods: Constructing next-generation meat, fish, milk, and egg analogs. **Comprehensive Reviews in Food Science and Food Safety**, 20(4): 4049-4100.

Muroya, S., M. Oe, I., Nakajima, K. Ojima, and K. Chikuni. 2014. CE-TOF MS-based metabolomic profiling revealed characteristic metabolic pathways in postmortem porcine fast and slow type muscles. **Meat science**, 98(4): 726-735.

Naito, M., T. Harumi, and T. Kuwana, 2015. Long-term culture of chicken primordial germ cells isolated from embryonic blood and production of germline chimaeric chickens. **Animal Reproduction Science**, 153: 50-61.

Pain, B., M. E. Clark, M. Shen, H. Nakazawa, M. Sakurai, J. Samarut, and R. J. Etches. 1996. Long-term in vitro culture and characterisation of avian embryonic stem cells with multiple morphogenetic potentialities. **Development, growth, and differentiation**, 122(8): 2339-2348.

Pain, B., P. Chenevier, and J. Samarut. 1999. Chicken embryonic stem cells and transgenic strategies. **Cells Tissues Organs**, 165: 212-219

PaphatPremsiri, A. 2017. **cell cycle, cell growth, and differentiation cell cycle, cell growth, and differentiation**. [Online] Available: https://www.scimath.org-/lesson-biology/item/7007-cell-cycle-cell-growth-and-differentiation- cell-cycle-cell-growth-and-differentiation.

Post, M. J. 2012. Cultured meat from stem cells: Challenges and prospects. **Meat science**, 92(3): 297-301.

Sartell, J. 2018. **Reading the germinal disk and breeding** [Online] Availed: https://-www.communitychickens.com/reading-germinal-disk-breed-ing/ (20 July 2021).

Smyth Jr, J. R. 1990. **Genetics of plumage, skin and eye pigmentation in chickens**.

Developments in Animal and Veterinary Sciences (Netherlands).

Sowcharoensuk, C. 2020. **Industry Outlook 2020-2022: Chilled, Frozen and Processed Chicken** [Online] Availed: https://www.krungsri.com/en/research/-industry/-industry-outlook/FoodBeverage/Frozen-Processed-Chicken/IO/io-frozen-processed-chicken (20 July 2021).

Steel, R. G., J. H. Torrie, and D. A. Dickey. 1997. **Principles and Procedures of Statistics: A Biological Approach**. McGraw-Hill.

Sundekilde, U. K., M. K. Rasmussen, J. F. Young, and H. C. Bertram. 2017. High resolution magic angle spinning NMR spectroscopy reveals that pectoralis muscle dystrophy in chicken is associated with reduced muscle content of anserine and carnosine. **Food chemistry**, 217: 151-154.

Suwansan, R. 2017. **Basic Cell Biology and Cancer Genetics: The cell cycle**. [Online] Available: https://w1.med.cmu.ac.th/obgyn/lecturestopics/topicreview/564-1/.

Tian, Y., M. Xie, W. Wang, H. Wu, Z. Fu, and L. Lin. 2007. Determination of carnosine in Black-Bone Silky Fowl (Gallus gallus domesticus Brisson) and common chicken by HPLC. **European Food Research and Technology**, 226(1): 311-314.

Trotter, J. A., and M. Nameroff. 1976. Myoblast differentiation in vitro: Morphological differentiation of mononucleated myoblasts. **Developmental Biology**, 49(2): 548–55.

Tummy. 2019. **The global food crisis "Meat from the lab" is not far off.** [online] source: https://www.marketingoops.com/tech-2/cultured-meat/ (22 August 2021).

USDA, F. 2020. **Livestock and Poultry: World Markets and Trade**. United States Department of Agriculture. Foreign Agriculture Service.

Van der Weele, C., P. Feindt, A. J. van der Goot, B. van Mierlo, and M. van Boekel. 2019. Meat alternatives: an integrative comparison. Trends in **Food Science and Technology**, 88: 505-512.

Walkinshaw, B. Piñero. 2017. **A brief history of fake meat**. [Online] Availed: https://psmag.-com/news/a-brief-history-of-fake-meat (22 August 2021).

Wang, Y., Y. Yang, D. Pan, J. He, J. Cao, H. Wang, and P. Ertbjerg. 2020. Metabolite profile based on 1H NMR of broiler chicken breasts affected by wooden breast

myodegeneration. **Food chemistry**, 310: 125852.

Wilailak, C. 2012. Air and noise pollution from animal farms. **Kasetsart Livestock Journal**, 39(153): 47-53.

Wu, Y., C. Ge, W. Zeng, and C. Zhang. 2010. Induced multilineage differentiation of chicken embryonic germ cells via embryoid body formation. **Stem cells and development**, 19(2): 195-202.

Xing, T., X. Zhao, X. Xu, J. Li, L. Zhang, and F. Gao. 2020. Physiochemical properties, protein and metabolite profiles of muscle exudate of chicken meat affected by wooden breast myopathy. **Food chemistry**, 316: 126271.

Yablonka-Reuveni, Z., and M. Nameroff. 1987. Skeletal muscle cell populations. Separation and partial characterization of fibroblast-like cells from embryonic tissue using density centrifugation. **Histochemistry**, 87(1): 27–38.

Yu, Q., X. Tian, L. Shao, X. Li, and R. Dai. 2019. Targeted metabolomics to reveal muscle-specific energy metabolism between bovine longissimus lumborum and psoas major during early postmortem periods. **Meat science**, 156: 166-173.

Zhu, M., M. Wang, Y. Shao, Y. Nan, H. T. Blair, S. T. Morris, and H. Zhang. 2021. Characterization of muscle development and gene expression in early embryos of chicken, quail, and their hybrids. **Gene**, 768: 145319.

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