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STUDY ON THE FEASIBILITY OF USING LIVESTOCK ADULT SERUM AS A FETAL BOVINE SERUM SUBSTITUTE FOR CULTURED MEAT

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I. INTRODUCTION

Cultured meat, which refers to edible meat obtained through cell proliferation without going through the process of raising livestock, has emerged as an alternative technology to solve the concerns and perceptions about animal welfare and the environmental impacts of the livestock industry. As its name suggests, FBS is the serum fraction of blood taken from normal bovine fetus harvested from healthy pregnant cows at slaughter. This accounts for the largest cost in producing cultured meat (O'Neill et al., 2021). Many researchers are attempting to replace FBS with other materials or develop serum-free media, and technology development to completely replace FBS or acquire FBS-like materials at low cost; an important challenge for the industrialization of cultured meat.
Slaughter by-products from cattle, pigs, and chickens, including blood, are mostly discarded but contain a variety of bioactive substances, such as hormones, growth factors, protein, amino acids, glutamine, vitamins, or iron. Although research is underway to replace FBS, its use is still significantly effective in cell culture. Efficient use of by-products generated during meat production will not only reduce waste disposal costs and environmental pollution while improving the added value of by-products but also help improve animal welfare by resolving the controversy over fetal blood collection. In addition, the development of FBS substitutes is expected to be used not only for mass production of cultured meat but also for various types of cell line cultivation or vaccine production, which will lead to a reduction in production costs. Therefore, the purpose of this study is to develop an FBS substitute using slaughter blood to reduce the reliance on bovine fetus and the cost of cultured meat production.

II. MATERIALS AND METHODS

2.1. Preparation of FBS and FBS substitute

The process of manufacturing FBS substitutes using slaughter blood from bovine, porcine, and chicken sources. Thawed serum was again subjected to two different centrifugation steps to remove unnecessary impurities. The first product (referred to as Raw) was the as-recovered serum. The second product had undergone sonication treatment (referred to as Sonication). To this end, the thawed sample was repeatedly processed an ultra sonicator for 5s on and 5s off for 5 to 20 min (KFS-250N, Korprotech, Seoul, Korea), during which time, the sample temperature did not exceed 40 °C. The third product (referred to as Inactivation) was obtained by heating the thawed sample.

2.2 Bovine myosatellite cell culture

Bovine muscle for cell isolation was from a 34-month castrated Korean cattle beef (Hanwoo) and provided by Chungbuk National University. The bovine myosatellite cell was suspended in basal media containing inactivated-FBS (20%) and P/S/A (1%) and incubated in a 1% collagen coated dish. During culture, the medium was replaced every 2 to 4 days. All treatment groups, including the control group, were supplemented with cell culture additives CAU-α® and CAU-β®. CAU-α® contains a mixture of lipid-rich bovine serum albumin and insulin-transferrin-selenium, while CAU-β® contains a mixture of three types of growth factors, including basic fibroblast growth factors. Differentiation of myosatellite cells can be induced with myogenic medium, and the composition of this medium is changed over two stages. Differentiation induction was started with DMEM containing P/S/A (1%) and horse serum (2%) and then replaced with DMEM containing P/S/A (1%) and horse serum (10%).

III. RESULTS AND DISCUSSION

• Bovine serum: The FBS substitute obtained from bovine blood most reliably replaced FBS and resulted in bovine muscle cell proliferation and differentiation.
• Porcine serum: The FBS substitute obtained from porcine blood was confirmed to have a cell proliferation effect, but its final differentiation efficiency was poor.

• Chicken serum: For the FBS substitute obtained from chicken blood, the onset of differentiation was faster than other treatments (commercial FBS, bovine serum), and although the amount of cells was not large, myosin heavy chain was produced similarly to the bovine serum substitute in the final product.
These results suggest that among the three FBS substitutes we developed, bovine serum and chicken serum have the potential to be effective in proliferating cells for cultured meat production and differentiating them into muscle.
FBS is by-product obtained during cattle slaughter and is collected from deceased pregnant cows under government approval. One of the reasons it is difficult to replace FBS, which comes from bovine fetus, may be because its exact composition remains unknown. While we developed an FBS substitute that cannot completely replace animal derived-materials, FBS substitutes extracted from livestock slaughter blood can effectively grow muscle satellite cells, contributing to reducing the cost of cultured meat production. However, the complete composition and mechanism of FBS and mature blood remain unknown, requiring further research for optimized media development.

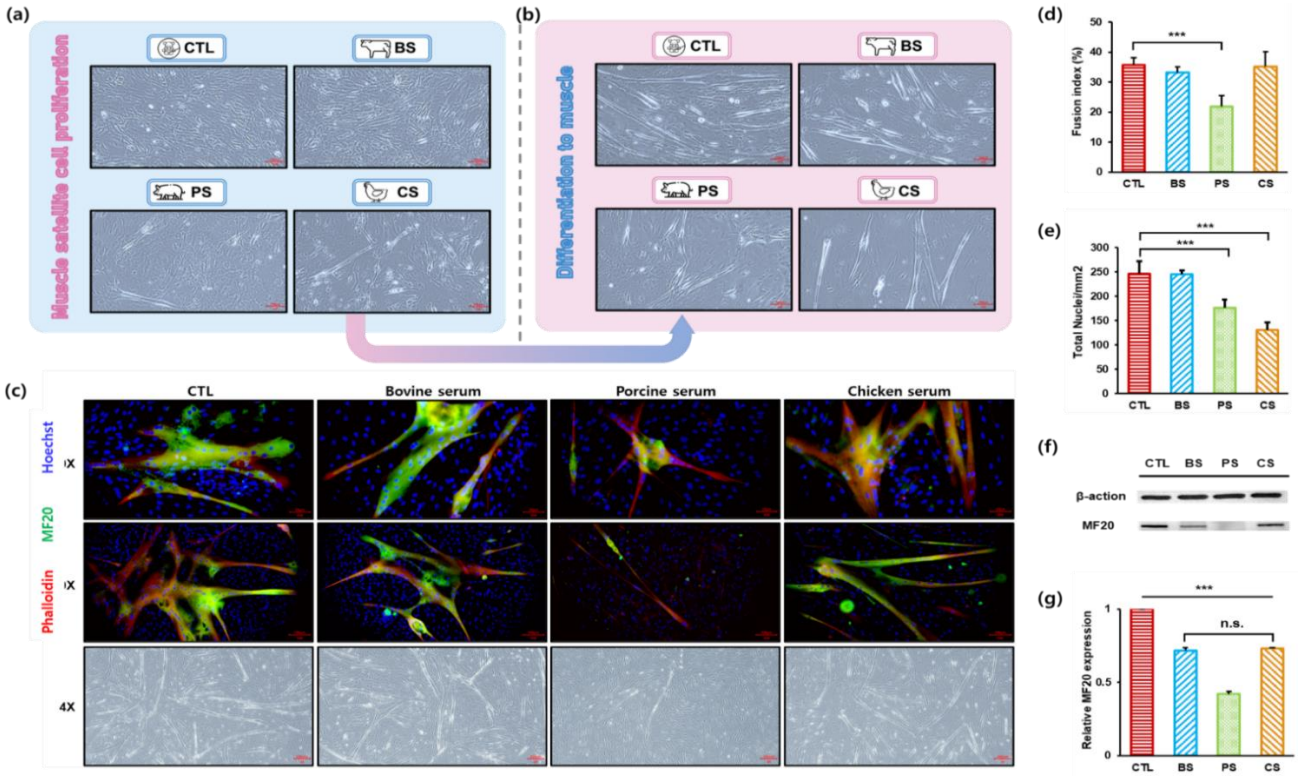


Figure 1. Analysis of cell morphology in response to myogenic differentiation in bovine myosatellite cells treated with FBS or FBS substitutes.

IV. CONCLUSION

For the first time, we found that FBS for cell cultivation could be replaced by semi-processed serum in bovine, porcine, and chicken slaughter blood. We reduced the cost of the medium composition (including FBS) by about 61% through the developed cultured meat. FBS substitutes (from bovine serum and chicken serum) successfully formed bovine myotubes and were found to be the most suitable FBS substitute when considering the production cost.

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