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Maximizing the potential of male layer embryos for cultivated chicken meat cell sourcing

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ABSTRACT

Background: This study explores the potential of discarded male layer embryos as a sustainable and non-GMO cell source for cultivated chicken meat production. The research aims to identify efficient methods for isolating muscle progenitor cells (MPCs) with high proliferative potential by conducting transcriptome analysis on thigh muscle tissues from both male and female chick embryos.

Methods: Transcriptome analysis was performed on the thigh muscle tissues of male and female chick embryos, aged 12-13 days, (n = 4 each), to investigate the gene expression profiles and identify strategies for efficiently isolating MPCs. This approach aims to pinpoint techniques that would allow for the selection of MPCs with optimal growth and proliferation capabilities.

Results: Using heatmap, hierarchical clustering, and multidimensional scaling (MDS), we found no significant sex-based differences in gene expression, except for the overexpression of the female-specific gene LIPBLL. The expression of muscle stem cell factors, including PAX3, PAX7, and other myogenic regulatory genes, showed no significant variation. However, to recover MPC-rich cells isolated from male thigh muscle, we found that by the pre-plating 7 stage, myogenesis-related genes, MYHs and MUSTN1 were minimally expressed, while the cell cycle arrest gene CDKN1A sharply increased.

Conclusions: Our findings suggest that simple cell isolation directly from tissue is a more scalable and efficient approach for cultivated meat production, compared to labor-intensive pre-plating methods, making it a viable solution for sustainable research and resource recycling.

Keywords: chicken cultivated meat, male layer embryos, MUSTN1, transcriptome analysis

INTRODUCTION

With the advancement of biotechnology and a growing focus on sustainability, research into cellular agriculture across various livestock species–including bovine, chicken, and porcine–has been gaining significant momentum (Yablonka-Reuveni et al., 1987; Doumit and Merkel, 1992; Post, 2014; Reiss et al., 2021; Messmer et al., 2022; Ju et al., 2023; Ock et al., 2023). The possibility of commercialization was first demonstrated in 2013 by Mark Post' s team through the creation of a cultured beef hamburger patty. Since then, numerous studies have been conducted, leading to significant advancements, including the recent approval by the United States Department of Agriculture

for the production and sale of cultivated chicken meat derived from chicken cells (Food and Drug Administration, 2023; Qiu, 2023).

Cellular agriculture employs a variety of cell sources, including adult stem cells such as muscle satellite cells, pluripotent stem cells (PSCs) like embryonic stem cells and induced pluripotent stem cells, as well as spontaneously and involuntarily immortalized fibroblasts. Each of these cell types presents specific advantages and disadvantages, which have been thoroughly explored in existing research. Historically, the focus of this field has been on muscle satellite cells or muscle progenitor cells (Zehorai et al., 2023). However, these cells face limitations such as low expandability and challenges in the collection process, hindering their wider application. While PSCs offer an alternative, they come with their own set of drawbacks, including difficulties in culture, limited accessibility, lengthy differentiation times, and the high cost of culture media. Moreover, immortalized fibroblasts, though useful, are not muscle cells and raise concerns regarding consumer safety, necessitating the development of appropriate countermeasures (Pasitka et al., 2023; 2024).

Chickens are increasingly favored for cultivated meat development due to their low fat content, high-quality protein, and broad consumer acceptance. In this study, we focused on muscle progenitor cells (MPCs) from male embryos of laying hens, selected for their ease of procurement, high productivity, safety, and potential to repurpose otherwise wasted resources. We aimed to identify skeletal muscle development characteristics through transcriptomic analyses of male and female embryos, using male embryos from typically discarded fertilized eggs. Additionally, we examined skeletal muscle-derived cells at various recovery times to determine the optimal timing for cell recovery and compared their growth potential to immortalized fibroblasts to understand their unique traits.

MATERIALS AND METHODS

Reagents and ethics statements

Unless stated otherwise, all reagents were obtained from Sigma-Aldrich (St. Louis, MO, USA) or Thermo Fisher Scientific (Frederick, MD, USA). This study's experiments were conducted in compliance with the Institutional Animal Care and Use Committee (IACUC) guidelines from the National Institute of Animal Science, Rural Development Administration, Republic of Korea (approval No. NIAS20222452).

Sample acquisition and processing

Fertilized eggs from SPF White Leghorn lines (Namdeok SPF, Seoul, Republic of Korea) were incubated following standard protocols (Ju et al., 2023). Ten embryos at embryonic day 12-13 were randomly selected for subsequent analysis. Thigh muscle tissue was aseptically excised using sterilized instruments. A portion of this tissue was used for primary MPCs culture, while genomic DNA was extracted from the remaining tissue for sex determination (He et al., 2019). Four male and four female embryos were subsequently randomly chosen for transcriptome analysis via RNA sequencing.

Isolation of musclec progenitor cells (MPCs)

To isolate MPCs from thigh muscles, single cells were first dissociated from the tissue following established protocols (Ju et al., 2023). From the resulting heterogeneous cell suspension, MPCs were selectively isolated using a pre-plating method (Wu et al., 2010) based on differences in cell attachment speed. The suspended cells were initially plated in a collagen-coated T75 flask (75 cm^2, Corning, NY, USA) and maintained in DMEM/F12 medium (Invitrogen-Gibco, Grand Island, NY, USA) supplemented with 20% fetal bovine serum (FBS, heat-inactivated), 10 ng/mL basic fibroblast growth factor and 1× Penicillin-Streptomycin at 37℃ in a humidified incubator with 5% CO_2 (pre-plating 1, pp1). After 1 hr of incubation, the supernatant containing the unattached cells was transferred to a new collagen-coated T75 flask and cultured (pp2). This process was repeated with the supernatant transferred to fresh collagen-coated T75 flasks after 2 hrs (pp3), 18 hrs (pp4), and subsequently every 24 hrs for pre-plates 5 through 7 (pp5, pp6, pp7).

Transcriptome analysis

For transcriptome analysis, total RNA was extracted from the samples, with each sample yielding more than 1 μg. Paired-end sequencing was performed using the Illumina NovaSeq platform (Illumina, Inc., San Diego, CA, USA). The reference genome used for analysis was GRCg6a. The sequencing protocol and software were based on the NovaSeq 6000 System User Guide (Document #1000000019358v02). Gene expression levels were con-

sidered significant with fold changes of ≥ 2 or ≥ 10 and raw *p*-values < 0.05. Differentially expressed gene (DEG) analysis was performed using gProfiler for gene ontology analysis. Hierarchical clustering and multidimensional scaling (MDS) were also performed. All analyses were conducted by Macrogen (Macrogen Inc., Seoul, Republic of Korea). Fold change values were calculated for selected transcripts based on the log2 ratio of their normalized expression levels relative to the control group.

Statistical analysis

Statistical analysis was performed using one-way ANOVA, followed by post-hoc tests to identify group differences ($p < 0.05$). Data are presented as means ± standard error of the mean (SEM) based on four independent biological replicates.

RESULTS

Transcriptome analysis of male and female thigh muscles and stage-specific cells

Whole transcriptome analysis of four male and four female thigh muscles revealed distinct transcript distributions compared to the negative control, DF-1 cells, as shown in Fig. 1A. No marked sexual dimorphisms were observed, but variations among individual samples were

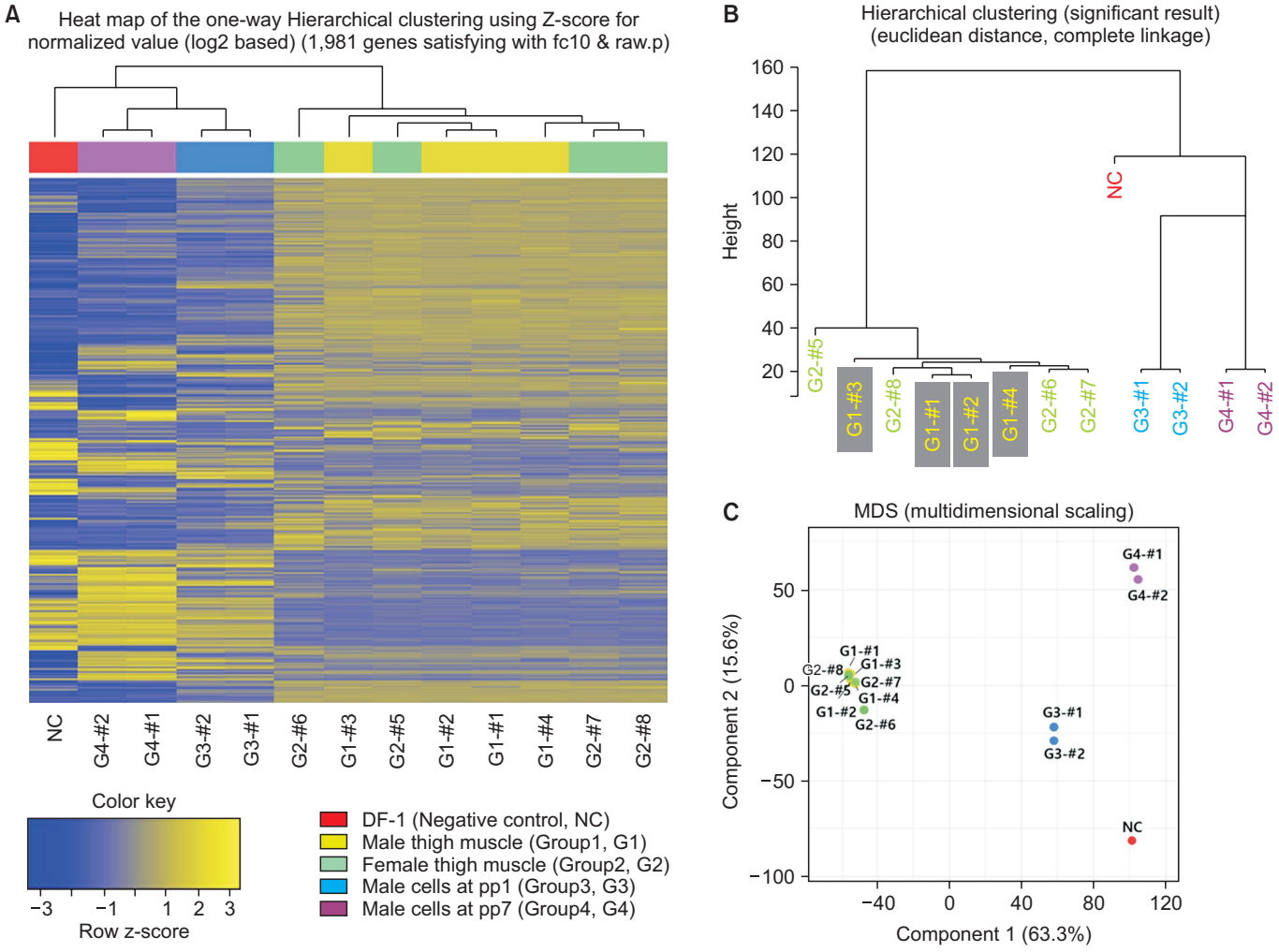

Fig. 1. Transcriptome analysis of muscle tissues and cells derived from thigh muscles of laying hen embryos. (A) Heatmap of transcriptome expression in thigh muscle tissues and derived cells. (B) Hierarchical clustering analysis among groups. (C) Multidimensional scaling (MDS) plot. Four groups were analyzed: males [Group 1 (G1), n = 4 (#1-4)], females [Group 2 (G2), n = 4 (#5-8)], and two derived cell types (pre-plating 1 and 7) from male thigh tissue [Group 3 (G3), n = 2 (#1-2)] and [Group 4 (G4), n = 2 (#1-2)]. DF-1 cells were used as the negative control [Group 5 (NC)].

detected. These findings were further validated through hierarchical clustering and MDS analyses (Fig. 1A-C). When examining the overall transcriptome distribution of cells at pp1 and pp7 stages recovered from male thigh muscles #1 and #2, pp1 cells exhibited greater similarity to thigh skeletal muscle. Hierarchical clustering and MDS analyses revealed that cells at the pp1 and pp7 stages shared numerous similarities but also exhibited distinct characteristics.

As shown in Fig. 2, the results were clearly confirmed by the Volcano Plot analysis of expression values between the two groups. While the transcriptomic profiles of male and female thigh skeletal muscles were nearly identical, distinct differential expression patterns emerged between cells at the pp1 stage and those at the pp7 stage, particularly at the 2-fold cut-off. Additionally, male thigh skeletal muscles exhibited significant differences in up- and down-regulated transcripts compared to the DF-1 control group.

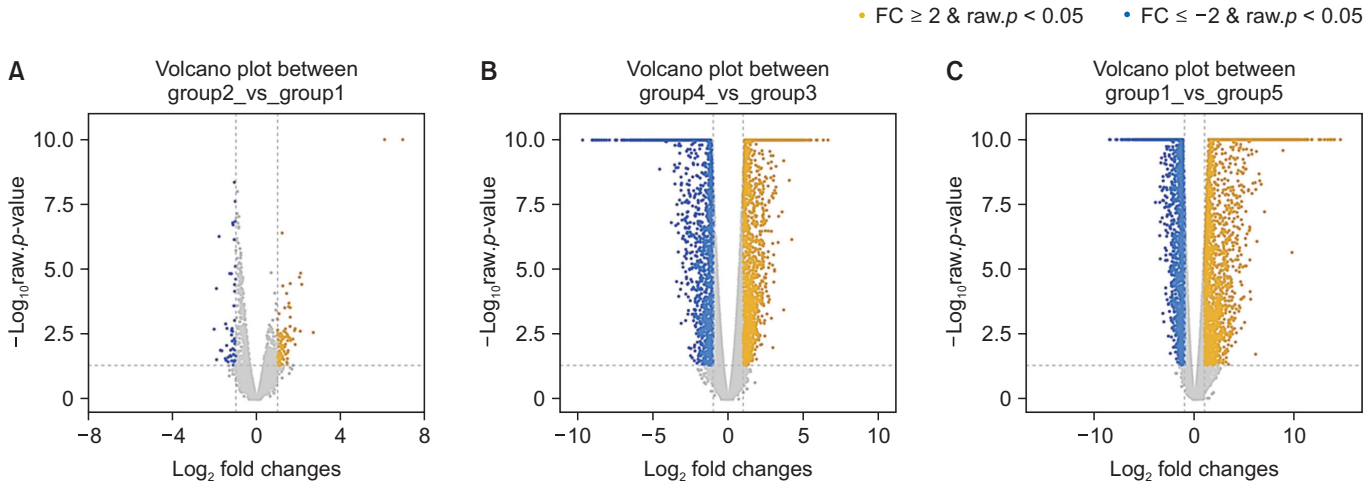

Fig. 2. Volcano plots comparing sex differences in thigh muscle tissues and cells from different pre-plating stages. (A) Male vs. female thigh muscle tissues; (B) pre-plating stage 1 vs. pre-plating stage 7 cells; (C) male thigh muscle vs. DF-1 cells. The fold change cutoff was set at 2, with significance tested at the $p < 0.05$ level.

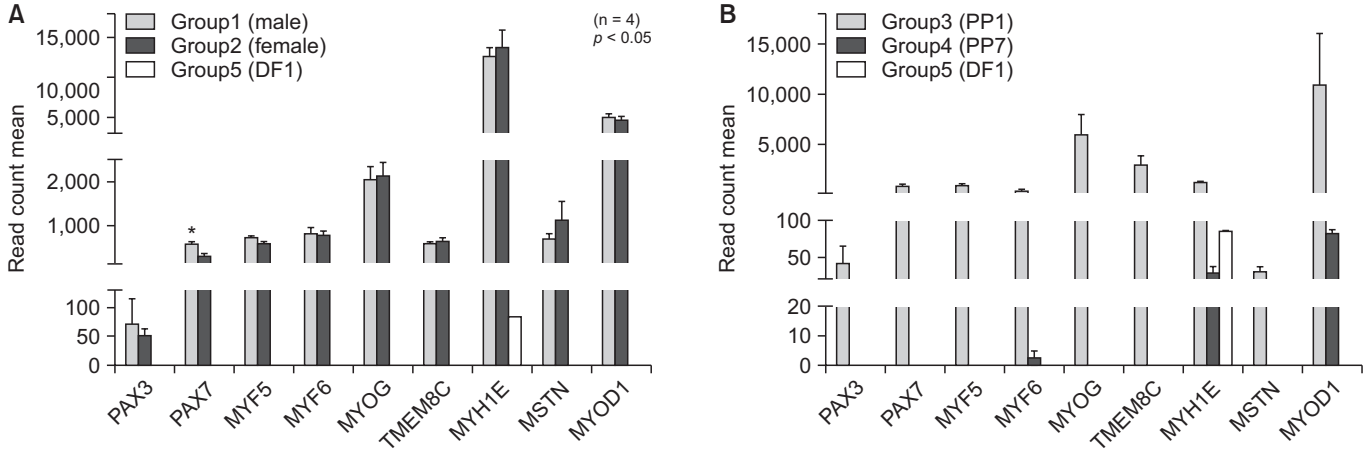

Fig. 3. Analysis of transcripts related to myogenic regulatory factors in thigh muscle tissues and cells from different pre-plating stages. (A) Means read counts ± standard error of the mean (SEM) in male (group 1) and female (group 2) thigh muscle tissues, and DF-1 cells (group 5). Significance between males and females was assessed at the $*p < 0.05$ level, with n = 4 for each group. (B) Mean read counts ± SEM for pre-plating stage 1 (Group 3), pre-plating stage 7 (Group 4) cells, and DF-1 cells (Group 5). Significance could not be assessed for pre-plating stages (pp1 vs. pp7) due to the limited number of repetitions (n = 2).

Expression of transcripts related to myogenic regulation factors (MRFs)

The expression levels of MRF-related transcripts were evaluated using read count values and compared between male and female thigh muscles (Fig. 3A). Only PAX7 showed sex-specific differences, but these did not meet the 2-fold significance threshold. In the negative control (DF-1 cells), no changes were observed except for MYH1E.

Under identical conditions, the expression of MRF-related transcripts was analyzed in male cells cultured at the pp1 and pp2 stages, focusing on differences in cell attachment rates (Fig. 1B). Although the small sample size limited statistical analysis, MRF-related transcripts was notably higher at the pp1 stage compared to the pp7 stage. At the pp7 stage, only 3 out of 9 transcripts–MYF6, MYH1E, and MYOD1–were detected at low levels. In the negative control DF-1 cells, only MYH1E was observed. Notably, the key MPC initiation factors PAX3, PAX7, and MYF5 were entirely absent at the pp7 stage.

Transcripts expression related to cell lifespan and proliferation

The expression levels of transcripts related to apoptosis, the cell cycle, and proliferation were analyzed (Table 1). In thigh muscle tissues, the pro-apoptotic BAK1 transcript showed relatively low expression, while most anti-apoptotic BCL2 family members were highly expressed, with the exception of BCL2L1. Additionally, no significant sex-specific differences were observed in the expression of these genes. Within the p53-p21-dependent growth arrest and apoptosis pathway, which plays a crucial role in apoptosis, four key genes (TP53BP1, TP53I3, TP53INP1, TP53INP2) were not activated, with the exception of TP53BP1, an upstream regulator of p53-related transcripts. Additionally, downstream components of the TP53 pathway, including p21, CDKN1A, and CDKN1AL, also showed no activation. No sex-related differences were observed in these pathways when comparing fold change values normalized between males and females. When examining the expression levels of MYC family transcripts, which are directly related to cell proliferation and lifespan, along with the telomerase synthesis factor transcript TERT, it was observed that MYC was down-regulated, while MYCBPAP, MYCL, MYCN, and MYCT1 showed elevated activity, particularly MYCT1. Although TERT exhibited a two-fold upregulation, this difference was not statistically significant due to individual variation.

Table 1. Expression of transcripts related to cell death and proliferation

Genes	Fold changes					
	Male/DF1 (G1/G5)	Female/DF1 (G2/G5)	Male/female (G1/G2)	PP1/DF1 (G3/G5)	PP7/DF1 (G4/G5)	PP1/PP7 (G3/G4)
BAK1	−2.47	−2.79	−1.13	1.08	−1.53	1.66
BCL2	6.43	5.67	1.13	3.94	5.83	−1.48
BCL2A1	7.88	7.66	1.03	12.34	148.09	−12.12
BCL2L1	−10.66	−9.77	−1.09	−4.04	−1.51	−2.67
BCL2L10	3.43	5.00	−1.46	2.79	1.66	1.68
BCL2L11	2.07	1.70	1.22	2.04	8.41	−4.13
BCL2L13	1.68	1.77	−1.05	1.53	3.06	−2.01
CDKN1A (P21)	1.89	2.80 (*p*.val > 0.05)	−1.48	68.62	550.62	−8.03
CDKN1AL (P21)	−2.54	−2.19	−1.16	−7.28	−1.63	−4.46
MYC	−3.90	−4.37	1.12	1.14	−1.31	1.49
MYCBPAP	2.79	2.55	1.09	−1.49	−1.59	1.07
MYCL	5.85	5.84	1.00	4.92	2.43	2.03
MYCN	11.98	12.88	−1.08	5.13	3.42	1.50
MYCT1	61.82	60.05	1.03	2.28	−2.61	5.96
TERT	2.30 (*p*.val > 0.05)	2.14 (*p*.val > 0.05)	1.08	4.79	6.27	−1.31
TP53BP1	−2.88	−3.36	1.17	−2.81	−4.33	1.54
TP53I11	498.42	516.41	−1.03	37.74	59.64	−1.59
TP53I3	−3.09	−1.40	−2.22	−1.05	1.69	−1.77
TP53INP1	1.81	1.68	1.08	−2.28	1.34	−3.07
TP53INP2	−2.37	−2.63	1.11	−1.48	1.20	−1.77

Table 2. Transcript expression of MYH family genes and hypertrophic growth marker in skeletal muscle cell maturation and differentiation

Genes	Fold changes					
	Male/DF1 (G1/G5)	Female/DF1 (G2/G5)	Male/female (G1/G2)	PP1/DF1 (G3/G5)	PP7/DF1 (G4/G5)	PP1/PP7 (G3/G4)
MYH1C	10,123.34	11,493.98	−1.13	11.87	1.24	9.56
MYH1E	231.32	234.17	−1.01	12.69	−2.28	29.00
MYH1G	11,971.52	12,068.16	−1.01	25.91	20.44	1.26
MYH10	1.12	1.02	1.10	1.44	−2.60	3.73
MYH11	45.47	44.89	1.01	6.75	1.12	6.06
MYH15	−11.52	−10.80	−1.07	2.56	−66.81	171.12
MUSTN1	167.22	369.16	−2.21	111.49	3.57	31.10

No sex-based differential expression was observed based on the fold change values of males normalized to females.

In cell analysis, the anti-apoptotic genes BCL2A1 and BCL2L10 were upregulated at the pp7 stage compared to pp1. However, when normalizing pp1 to pp7, both genes showed significant downregulation, with fold changes of -12.12 and -4.13, respectively. Unlike in tissues, the cell cycle arrest gene CDKN1A (p21) was significantly upregulated in cells, showing a 550.62-fold increase at the pp7 stage compared to pp1. This upregulation corresponded to a fold change of -8.03 when normalizing pp1 to pp7, indicating enhanced cell cycle arrest at the pp7 stage. In contrast, TP53I11 also increased, but to a lesser extent than in tissues. Furthermore, the four MYC family genes that were upregulated in tissues exhibited more modest increases in cells, particularly at the pp7 stage, with MYCL and MYCT1 displaying fold changes of 2.03 and 5.96, respectively, when normalized to pp7.

Transcript analysis of genes related to cell lifespan and proliferation revealed distinct patterns between tissues and cells. While no significant sex-specific differences were found in chicken thigh muscle, aside from a female-specific gene, cells nearing the final pre-plating stages showed notable changes: anti-apoptotic genes were upregulated, cell cycle arrest genes were strongly upregulated, and MYC family genes were downregulated.

Expression of MYHs and MUSTN1 involved in skeletal muscle maturation

The involvement of the Myosin Heavy Chain (MYH) family in muscle contraction was analyzed, and the findings are summarized in Table 2. Isoforms MYH1, MYH1C, MYH1E, and MYH1G were strongly expressed in thigh muscle, with no significant differences observed between sexes. The MYH11, typically expressed in smooth muscle, was present at lower levels compared to skeletal muscle but remained detectable in the tissue and in pp1 stage cells. MYH10, a non-muscle myosin, was expressed at levels comparable to DF-1 cells but showed a decrease in pp7 stage cells. In cellular analysis, the MYH1 isoforms and MYH15 were upregulated at the pp1 stage compared to the pp7 stage, a pattern that was confirmed when pp1 was normalized with pp7, except for MYH1G. The musculoskeletal, embryonic nuclear protein 1 MUSTN1, which is involved in muscle hypertrophy, showed a 31.10-fold down-regulation in pp7 cells compared to pp1 cells after normalization. This indicates a sharp decline in its role in muscle development. In conclusion, pp1 stage cells contain a higher number of MYH family genes and MUSTN1 associated with muscle development than pp7 stage cells.

DISCUSSION

In our study, we aimed to advance the development of scalable cell sources and promote resource recycling in chicken cultivated meat research by focusing on male laying hen embryos. We conducted a comprehensive analysis of the genetic characteristics and potential of these embryos as a cell source, examining genes associated with myogenic regulatory factor pathways and cell longevity at both tissue and cellular levels. The findings revealed no significant differences in differential gene expression between male and female embryos, aside from sex-related genes. Furthermore, cell separation methods commonly used for slow-adhering MPCs appeared to function differently when applied to highly proliferative embryonic cells.

In the primary embryonic thigh muscle, the overall transcriptome showed remarkable similarity between males and females, with the exception of the female-specific gene Nipped-B homolog-like (NIPBLL), which

was upregulated by 124.32-fold compared to male levels (Rallabandi et al., 2019). A total of 22 genes were identified with a three-fold cut-off; of these, 8 were uncharacterized transcripts, while the others were not directly related to muscle development. These findings were further validated through Heatmap, MDS, and Volcano plot analyses. Contrary to expectations, these results suggest that thigh muscles of male embryos do not possess any particular advantage in terms of skeletal muscle development, although this may be a characteristic specific to laying hens.

Cells derived from thigh muscle exhibited distinct distributions across pre-plating stages, diverging from the typical outcomes expected when isolating MPCs, such as myoblasts and muscle stem cells, from adult tissues (Wu et al., 2010). Prior researches have suggested that the purity of MPCs with slower adhesion capacity increases as pre-plating stages advance, but our MDS and hierarchical clustering analyses did not corroborate this finding (Wu et al., 2010). These differences could be attributed to species variations or differences in tissue collection sites (Wu et al., 2010; Furuhashi et al., 2021). In the embryonic stage, satellite cells are actively proliferating, whereas in adults, they decrease in number and are mostly quiescent, only activating in response to conditions like muscle damage (Mohammadabadi et al., 2021). For cultivated meat, scalability is key to success, requiring large numbers of cells. As over 85% of skeletal muscle cells are myocytes (Furuhashi et al., 2021), all stages of muscle cell development–from satellite cells to myotubes–can be harnessed, unless the study specifically focuses on muscle metabolism or disease.

This study comprehensively analyzes the proliferation potential of cell types derived from thigh muscle, with a particular focus on pp7 cells. Our findings reveal that pp7 cells exhibit significantly decreased proliferation capacity primarily due to the overexpression of anti-apoptotic factors and the cell cycle-arrest gene p21. This suggests that pp7 cells are less likely to undergo cell division, a crucial factor for their potential use in muscle growth and regeneration. Furthermore, our analysis of muscle maturation and differentiation demonstrates a striking 29-fold reduction in the expression of MYH1E, a key member of the MYH1 family. While other MYHs were expressed at similar or lower levels compared to DF-1 cells, the downregulation of MYH1E suggests impaired muscle maturation and hypertrophy. Additionally, we investigated the role of MUSTN1, a gene linked to skeletal muscle hypertrophy (Hadjiargyrou, 2018; Mohammadabadi et al., 2021). In pp7 cells, MUSTN1 was significantly downregulated, correlating with a marked suppression of skeletal muscle stem cell proliferation. This further limits the ability of pp7 cells to participate in myogenesis, reinforcing our conclusion that their capacity for muscle regeneration is severely restricted. In conclusion, our results highlight the limitations of pp7 cells as a potential cell source for muscle regeneration and cultivated meat production. These cells exhibit reduced proliferation, impaired myogenesis, and a suppressed capacity for skeletal muscle stem cell proliferation. Collectively, these findings indicate that pp7 cells possess both limited proliferative ability and a diminished potential for muscle growth. As a result, the utility and cost-effectiveness of pp7 cells as a source for cultured meat production are significantly compromised. This underscores the importance of selecting cell lines with higher regenerative and proliferative capacities for efficient and sustainable production of cultivated muscle tissue.

CONCLUSION

From the perspective of scalability in cultivated chicken meat production, this study demonstrates that using male layer embryos as a cell source offers several advantages. These include a rich supply of muscle cells, improved economic efficiency through reduced labor and culture medium requirements, and the convenience of harvesting cell sources from otherwise discarded materials. Furthermore, the findings confirm that MPCs from these embryos possess high proliferative potential, eliminating the need for the multi-step separation processes typically required for adult cells.

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Ethical Approval: This study's experiments were conducted in compliance with the Institutional Animal Care and Use Committee (IACUC) guidelines from the National Institute of Animal Science, Rural Development Administration, Republic of Korea (approval No. NIAS20222452).

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Consent to Publish: Not applicable.

Availability of Data and Materials: Not applicable.

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REFERENCES

Doumit ME and Merkel RA. 1992. Conditions for isolation and culture of porcine myogenic satellite cells. Tissue Cell 24:253-262.

Food and Drug Administration. 2023. Human food made with cultured animal cells. https://www.fda.gov/food/food-ingredients-packaging/human-food-made-cultured-animal-cells

Furuhashi M, Morimoto Y, Shima A, Nakamura F, Ishikawa H, Takeuchi S. 2021. Formation of contractile 3D bovine muscle tissue for construction of millimetre-thick cultured steak. NPJ Sci. Food 5:6.

Hadjiargyrou M. 2018. Mustn1: a developmentally regulated pan-musculoskeletal cell marker and regulatory gene. Int. J. Mol. Sci. 19:206.

He L, Martins P, Huguenin J, Van TN, Manso T, Galindo T, Gregoire F, Catherinot L, Molina F, Espeut J. 2019. Simple, sensitive and robust chicken specific sexing assays, compliant with large scale analysis. PLoS One 14:e0213033.

Ju WS, Seo K, Lee BR, Park MR, Lee MG, Byun SJ, Yang H, Kim Y, Ock SA. 2023. Potential application of muscle precursor cells from male specific-pathogen-free (SPF) chicken embryos in in vitro agriculture. Animals (Basel) 13:1887.

Messmer T, Klevernic I, Furquim C, Ovchinnikova E, Dogan A, Cruz H, Post MJ, Flack JE. 2022. A serum-free media formulation for cultured meat production supports bovine satellite cell differentiation in the absence of serum starvation. Nat. Food 3:74-85.

Mohammadabadi M, Bordbar F, Jensen J, Du M, Guo W. 2021. Key genes regulating skeletal muscle development and growth in farm animals. Animals (Basel) 11:835.

Ock SA, Seo KM, Ju WS, Kim YI, Wi HY, Lee P. 2023. Effect of serum and oxygen on the in vitro culture of Hanwoo Korean native cattle-derived skeletal myogenic cells used in cellular agriculture. Foods 12:1384.

Pasitka L, Cohen M, Ehrlich A, Gildor B, Reuveni E, Ayyash M, Wissotsky G, Herscovici A, Kaminker R, Niv A, Bitcover R, Dadia O, Rudik A, Voloschin A, Shimoni M, Cinnamon Y, Nahmias Y. 2023. Spontaneous immortalization of chicken fibroblasts generates stable, high-yield cell lines for serum-free production of cultured meat. Nat. Food 4:35-50.

Pasitka L, Wissotsky G, Ayyash M, Yarza N, Rosoff G, Kaminker R, Nahmias Y. 2024. Empirical economic analysis shows cost-effective continuous manufacturing of cultivated chicken using animal-free medium. Nat. Food 5:693-702.

Post MJ. 2014. Cultured beef: medical technology to produce food. J. Sci. Food Agric. 94:1039-1041.

Qiu L. 2023. U.S. Approves the sale of lab-grown chicken. The New York Times. https://www.nytimes.com/2023/06/21/us/lab-grown-meat-sale-approval.html

Rallabandi HR, Yang H, Jo YJ, Lee HC, Byun SJ, Lee BR. 2019. Identification of female specific genes in the W chromosome that are expressed during gonadal differentiation in the chicken. Korean J. Poult. Sci. 46:287-296.

Reiss J, Robertson S, Suzuki M. 2021. Cell sources for cultivated meat: applications and considerations throughout the production workflow. Int. J. Mol. Sci. 22:7513.

Wu X, Wang S, Chen B, An X. 2010. Muscle-derived stem cells: isolation, characterization, differentiation, and application in cell and gene therapy. Cell Tissue Res. 340:549-567.

Yablonka-Reuveni Z, Quinn LS, Nameroff M. 1987. Isolation and clonal analysis of satellite cells from chicken pectoralis muscle. Dev. Biol. 119:252-259.

Zehorai E, Maor-Shoshani A, Molotski N, Dorojkin A, Marelly N, Dvash T, Lavon N. 2023. From fertilised oocyte to cultivated meat - harnessing bovine embryonic stem cells in the cultivated meat industry. Reprod. Fertil. Dev. 36:124-132.