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Original Article

Characterization of Bovine Adipose-derived Stem Cells from Two Isolation Methods

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Abstract: Adipose-derived stem cells (ADSCs) have great potential in regenerative medicine and tissue engineering. Especially, bovine ADSCs play an important role in the quality of artificial meat. In this study, we investigated the characterization of Zebu hybrid cattle's ADSCs isolated by enzymatic treatment and explant culture methods from the fat pad of cattle hoof. Characterization of ADSCs was evaluated by the self-renewal and specific marker expression. The self-renewal capacity was examined by population doubling time (PDT) and growth kinetics of ADSCs. Specific markers concluded Cluster of Differentiation 73 (CD73), CD90, CD44, CD45, CD29 were analysised by polymerase chain reaction (PCR). The PDT of ADSCs from the enzymatic treatment method was significantly higher than from the explant culture method. Besides, when comparing the level of proliferation and stability of adipose stem cells, we found a difference in culture efficiency between the two methods. In addition, the expression of markers specific to adipose stem cells shows that cells from adipose tissue using two isolation methods are quite stable in morphology and the markers are maintained in expression over time. These results can have several efficient advantages in *in vitro* production of meat alternatives.

Keywords: Adipose derived stem cells, bovine, isolation methods, characterization.

1. Introduction

Mesenchymal stem cells (MSCs) have been widely studied for their cell biology, clinical potential and tissue engineering [1]. MSCs can be isolated from diverse tissues. However, adipose tissue and bone marrow are the most common and longest-applied adult source tissues for human MSCs [1]. Adipose tissue-derived mesenchymal stem cells (AT-MSCs) or adipose-derived stem cells (ADSCs) have several benefits over bone marrow-derived

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mesenchymal stem cells (BM-MSCs) [2]. ADSCs can be obtained easily with higher yield (10% nucleated cells from ADSCs versus 0.001 – 0.01% of BM-MSCs) through a minimally invasive and painless procedure [3, 4]. Moreover, ADSCs show higher culture period and proliferation capacity [5, 6]; and may be better candidates for allogenic transplantation than BM-MSCs [7]. Therefore, the clinical approaches involving ADSCs are currently increasing.

Recently, ADSCs have been noticed as a cell source for artificial meat production of domesticated animals, especially cattle [8]. Artificial meat is cultured meat produced by tissue engineering techniques to create meat from animal cells without sacrificing the animals. This technology has several advantages such as pathogen control, decreasing agricultural land and animal slaughter, and reducing antibiotics use and environmental population [9, 10]. Meat fat is a key determinant of taste, texture, smell, and tenderness [11]. For example, Japanese Wagyu meat shows its marble structure and contains more than 50% fat [12]. The fat content of cultured meat can come from added lipids (fatty acid molecules) or lipids contained in adipocytes [13]. Bovine ADSCs can be isolated, expanded and finally differentiated into adipocytes for produced artificial meat.

In humans, characteristics of ADSCs are well indicated. The International Society for Cellular Therapy (ISCT) and the International Federation for Adipose Therapeutics and Science (IFATS) specify three minimal criteria for defining ADSCs: (1) cells must be plastic-adherent; (2) they must express CD73, CD90, and CD105 and lack the expression of CD14, CD11b, CD45, CD19, CD79, and human leukocyte antigen-DR (HLA-DR); and (3) they must have the potential to differentiate into preadipocytes, chondrocytes, and osteoblasts [14, 15]. However, the expression of bovine ADSCs surface marker phenotypes can vary depending on the isolation and culture method. Bovine ADSCs collected by enzymatic treatment method expressed CD90, CD105, and CD79, but did not express CD45, CD34, and CD73 [16]. Bovine ADSCs obtained from collagenase treatment showed expression of Vimentin, CD49d and CD13 antigens and lack of CD34 antigen (Ren et al., 2010). However, bovine ADSCs from the explant culture method expressed CD73 and CD90 and lack expressed CD45 and CD34 [17].

In this study, we compare the characteristics of bovine ADSCs obtained from enzymatic treatment and explant culture method.

2. Materials and Methods

2.1. Reagents

All chemical compounds and cell culture reagents were purchased from Sigma-Aldrich Corporation (USA) and Thermo Fisher Scientific (USA), respectively, and cell culture ware was obtained from SPL Life Science (South Korea) unless stated otherwise.

2.2. Bovine ADSCs Isolation and Culture

A zebu hybrid cattle leg was collected from local slaughter house in Hanoi, Vietnam and transported to laboratory within 2 hour after slaughter. After cleaning, the leg was put on a sterilized tray with the upside-down hoof. The hoof was sterilized with 70% ethanol. The interdigital fat pads were cut using the sterilized scalpel, scissor and forceps and put on a petri dish. Then the adipose tissue dish was washed three times with Phosphate-Buffered Saline (PBS) supplemented with 100 U/ml penicillin, 100 µg/ml streptomycin and then minced carefully into 1-3 mm pieces. With the explant culture method, the pieces were plated into 35 mm dishes and covered with 2 ml of Dulbecco's Modified Eagle Medium (DMEM) high glucose (HG) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 U/ml penicillin, 100 µg/ml streptomycin and then incubated at 37 °C in a humidified atmosphere of 5% CO_2 for 9-11 days. The culture medium was replaced every 3 days. When the visible colonies were observed, cells were sub-cultured into 60 mm dishes at the density of 10^4 cells/cm^2.

With the enzymatic treatment method, the minced tissues were incubated with 0.05% trypsin/0.02% EDTA for digestion for 3 hour at 37 ^{o}C. Following incubation, an equal volume of DMEM-HG supplemented with 10% FBS was added to stop the digestion. The solution was filtered by a 70 µm cell strainer and then centrifuged at 3000 rpm for 5 min. The cell pellet was resuspended in a growth medium and plated in 35 mm dishes and incubated at 37 ^{o}C and 5% CO_2. The culture medium was changed 24h after seeding and every 3-4 days thereafter. When cells reached 80 – 90% confluence, cells were passaged and replated in 35 mm dishes at a density of 2 x 10^4 cells/cm^2.

2.3. Bovine ADSC Proliferation

Cells from explant culture and enzymatic digestion methods at P2 were plated in 96-well plates at a density of 4000 cells/cm^2 and cultured in DMEM HG medium supplemented with 10% FBS. The growth of cells was assessed over an 8-day period. The cell count was obtained from day 1 to day 8. Cells were trypsinized for 5 min then the neutralized by adding equal volume of culture medium. The cell suspension was obtained and centrifuge to obtain cells. The cells were then counted using a hemacytometer and Fluorescein Diacetate/ Propidium Iodide (FDA/PI) staining. Mixture of FDA and PI are freshly prepared: 1 µl stock FDA (0.1 mg/ml in acetone), 1 µl stock PI (0.5 mg/ml in PBS), and 100 µl PBS. The cells were stain 5 minute with FDA/PI solution and loaded onto hemocytometer. Then, stained cells were observed under a fluorescence microscope (a Nickon ellipse 90i combined with a Nickon DSFi2 camera) to evaluate live and dead cells. Cell population doubling time (PDT) at P2 was evaluated on day 5. PDT was calculated using the following formula: PDT = (CT x ln2)/ln(Nf/Ni) where, CT: was the cell culture time (hours), Ni: was initial and Nf: was the final cell numbers [18].

2.4. Specific-marker Analysis by Reverse Transcriptase-polymerase Chain Reaction (RT-PCR)

First, total RNA was extracted from the cells from explant culture and enzymatic digestion methods at P3 using TRIzol® Regent according to the manufacture's instructions. The resulting RNA concentration was determined using a NanoDropTM Lite Spectrophotometer (Thermo Fisher Scientific, USA). Then cDNA was synthesized from total RNA (1 µg) using the GostriptTM Reverse Transcription system kit (A5001, Promega, USA) following the manufacture's instruction (oligo(dT)15 primer was used). Afterward, cDNA concentration was determined using a NanoDrop machine. Then polymerase chain reactions (PCR) were carried out with cDNA as template, master mix (My TagTM Mix kit, BIO-25042, Meridian Bioscience, USA), primers shown in Table 1, and the amplifications were performed in Techne Genius FGEN05TP (Techne, USA). PCR was conducted for 35 cycles (95 °C for 30s, amplification temperature (Ta, Table 1) for 30s, 72 °C for 30s). Expression was analyzed followed by 2% agrarose (Genaxxon, Germany) gel electrophoresis and ethidium bromide staining. The product size was determined by 100bp HyperLadderTM (BIO-33056, Bioline, Meridian Bioscience or 1kb HyperLadderTM (BIO-33053).

2.5. Statistical Analysis

All experiments were repeated three times. All data were presented as mean ± standard error of the mean (SEM) from three separate experiments. All statistical analyses were carried out in Minitab statistical software using one-way ANOVA, and the Tukey post hoc test was used as a post hoc test. $p < 0.05$ were considered statistically significant.

Table 1. Specific primer pairs used for RT-PCR

Gene	Primer sequence (5' – 3')		Ta (^{o}C)	Size (bp)
NT5E (CD73)	F:	AGGTGTCACAAGAAGAGAACCT	55	173
	R:	TCAACCTTGTCTTTCCTCTGGA		

PTPRC (CD45)	F:	ATATTTTGCGCGTTGTTCAACC	56	128
	R:	AATTGGTATTGCTCGAAGGTGG		
THY1 (CD90)	F:	TTCGTCTAGACTGCCATCATGA	56	183
	R:	TGAAGTTGGACAGGTAGAGGAC		
CD44	F:	TCAAGTATCATCTCAGCAGGCT	55	266
	R:	TCTCCGTAAGCACTGGTACTAC		
ITGB1 (CD29)	F:	GTGTATACAAGCAGGGCCAAAT	56	265
	R:	TGATCGTAACTGCAGAACCAAC		
GAPDH	F:	GATAGCCGTAACTTCTGTGCTG	56	255
	R:	TTTCCATTGATGACGAGCTTCC		
Actin (ACTB)	F:	ATGCTTCTAGGCGGACTGTTAG	55	174
	R:	ACCTTCACCGTTCCAGTTTTTA		

3. Results

3.1. Bovine ADSCs Isolation and Culture

Fat tissue in the interdigital region of Zebu hybrid cattle's leg showed light yellow with little vascularization (Figure 1). In the explant culture group, some cells grew from the collected fat piece. These cells have elongated axonemal shapes adhered to the surface of the culture dish (Figure 2A, 2B). These cells expanded to form cell clusters after being cultured for up to six days (Figure 2C). After reaching 80% confluence after 9-11 days, these cells were passaged. The cells at the first passage (P1) retained a fibroblasts-like shape (Figure 2D).

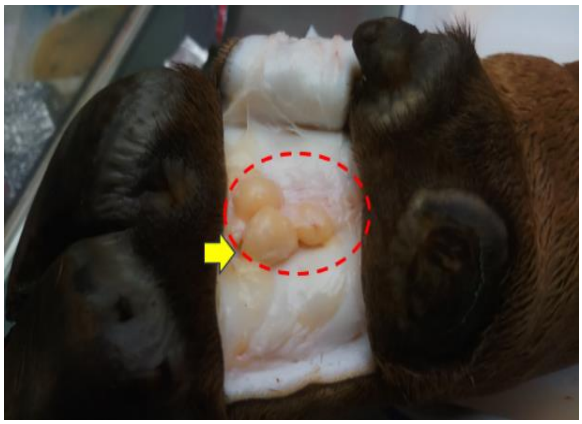

Figure 1. Fat tissue in the interdigital region of Zebu hybrid cattle's leg.

In the trypsin digestion group, cells attached to the plastic dishes and grew fast. After 3 days, cells reached 30% confluence (Figure 2E) and became confluence at day 6. Cells were subcultured and reached 80% confluence after 3 days (Figure 2F). These cells' morphology is similar to that of the explant culture group.

In the primary culture, the duration for cells in the explant culture reached 80% confluence, which was longer than for cells in the digestion method, which reached confluence (9-11 days vs 6 days).

3.2. Specific-marker Analysis by RT-PCR

Cells from both methods at P2 were analyzed with specific markers of MSCs, and the results were shown in Figure 3. Cells from explant culture and digestion method were positive with reference gene (GAPDH, ACTIN), specific markers of MSCs (CD29, CD90, CD73, CD44), and negative with the marker of hematopoietic stem cells (CD45). These results indicated that cells from both groups are ADSCs.

3.3. Bovine ADSCs Proliferation

After staining with FDA/PI, live cells were counted (green cells, Figure 5). Results of PDT of ADSCs from both methods were shown in Figure 6. PDT of cells from the explant method was significantly higher than those from the trypsin digestion medthod (24.76 ± 0.29 hour vs 22.01 ±

0.47 hour; $p < 0.05$). The growth kinetics results of cells from both methods were shown in Figure 7. However, from day 3–8, the number of cells significantly differed between the two methods ($p < 0.05$). After 8 days, the cell number of the trypsin group were 1.9 times higher than these cells isolated by the explant culture method.

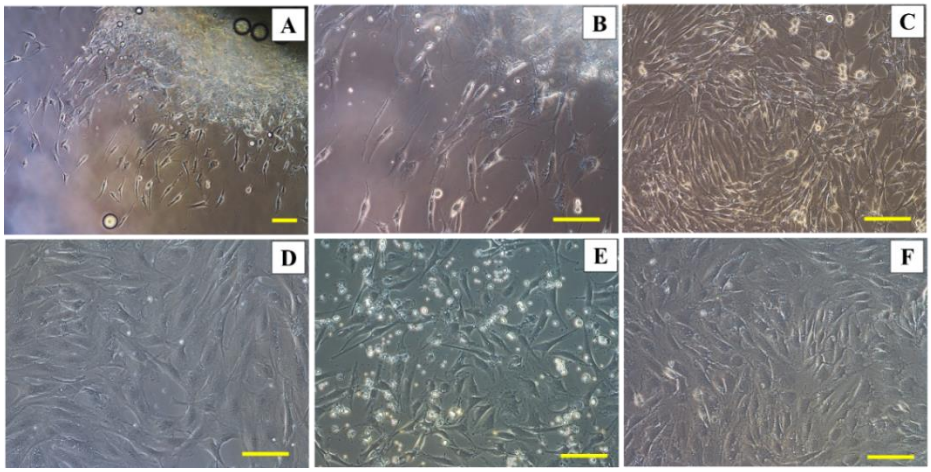

Figure 2. ADSCs from explant culture (A-D) and enzyme treatment method (E, F). Cells grew from adipose fragment after culturing 3 days at 100x (A), 200x (B) and 6 days at 200x (C). Cells collected by trypsin proliferated at day 3. Cells at day 3 of first passage (P1) from explant culture (D) and trypsin treatment (F). Scale bar, 100 µm.

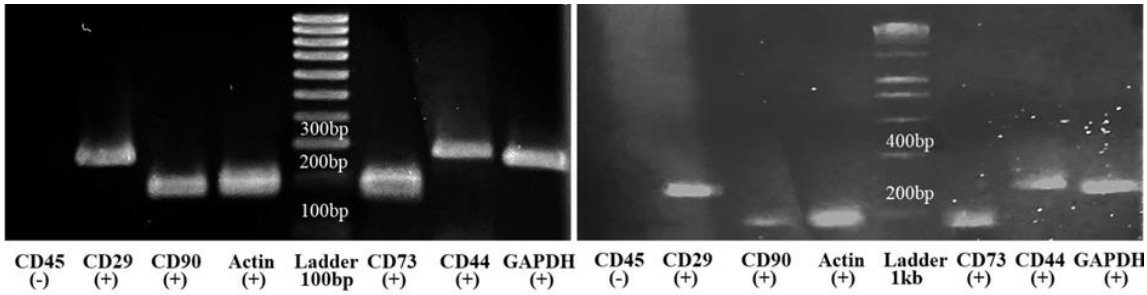

Figure 3. Gel electrophoresis of ADSCs from explant culture (A) and trypsin treatment (B) at day 5 of P2.

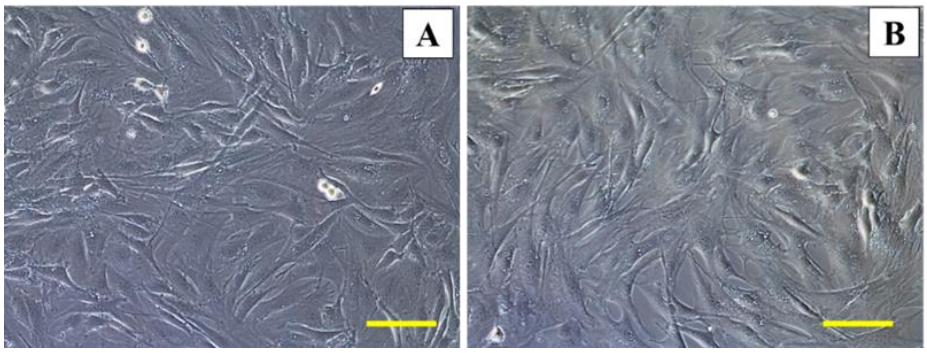

Figure 4. ADSCs from explant culture (A) and trypsin treatment (B) at day 5 of P2 with magnification 200x. Scale bar, 100 µm.

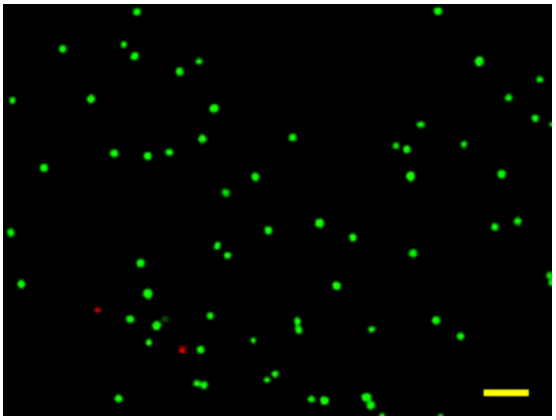

Figure 5. Images of ADSCs from the trypsin digestion method stained with FDA/PI. Green: live cells, red: dead cells. Magnification 100x. Yellow scale = 100 µm.

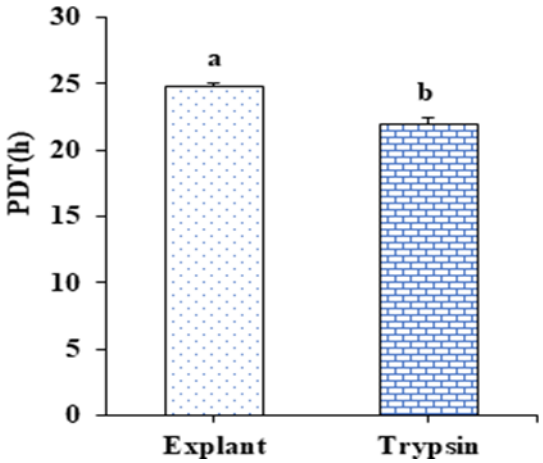

Figure 6. PDT of ADSCs from explant culture and trypsin digestion. Means with different lowercase letters are significantly different at $P < 0.05$.

4. Discussion

In this study, both isolation methods yielded cell populations that adhered to the surface of the cultured plastic dish and had cell morphology similar to fibroblasts. This result is similar to the morphology of bovine ADSCs in previous studies [19-21].

The CD73, CD44, and CD90 are markers of MSCs in general and ADSCs in particular. In this study, stem cells derived from bovine adipose tissue also expressed these specific markers. This result is similar to studies on bovine MSCs. Bovine MSCs derived from various tissues have been shown to be positive for adhesion-related mesenchymal markers such as CD29, CD166, CD105, surface enzymes such as CD73, receptors such as CD44, and glycoproteins such as CD90 [16, 22-24]. In studies of bovine ADSCs, the authors also showed that bovine ADSCs had a fibroblastic morphology and were positive for CD90 [16, 25]. However, Silva et al., [25] showed that these ADSCs were positive for CD73, CD105, negative for CD45, and CD44 while Sampaio et al., [16] reported that the ADSCs obtained were negative for CD45, CD24, and CD73. These differences may be due to differences in the ADSC isolation and culture methods. In this study, the ADSCs obtained were negative for CD45 and positive for CD73, CD29, CD44, and CD90.

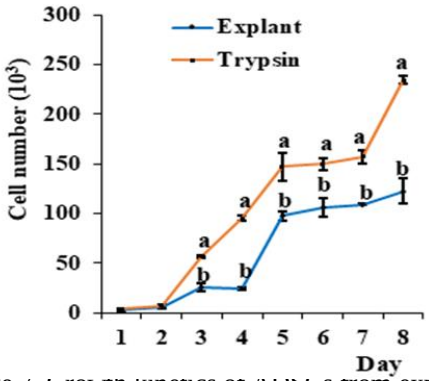

Figure 7. Growth kinetics of ADSCs from explant culture and trypsin digestion. Means with different lowercase letters are significantly different at $P < 0.05$.

In this study, ADSCs cells isolated by the enzymatic method had a higher proliferation potential than those isolated by the explant culture method. This results are consistent with the results of Zhu et al., [21]. The doubling time of Zebu hybrid bovine ADSCs in both methods in this study is short (less than 30 hour). This result is higher than the doubling time of cell numbers in P1-P4 in the study of Zhao et al., [20], PDT ranges from 16.44 ± 0.33 to 18.66 ± 0.11 hour and is similar to the PDT of ADSCs in P5 (22.06 ± 0.32 hour).

5. Conclusion

Zebu hybrid bovine ADSCs can be isolated by both explant culture and trypsin digestion methods. These cells have the characteristics of MSCs. These cells had fibroblast morphology, attached to plastic surfaces, and expressed the specific markers of MSCs (positive for CD73, CD90, CD44, CD29 and negative for CD45). However, cells isolated by trypsin digestion can proliferate faster than cells from the explant culture method.

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References

[1] M. F. Pittenger, D. E. Discher, B. M. Péault, D. G. Phinney, A. I. Caplan, Mesenchymal Stem Cell Perspective: Cell Biology to Clinical Progress, NPJ Regen. Med. Vol. 4, 2019, pp. 1-15 https://doi.org/10.1038/s41536-019-0083-6.

[2] J. Zhang, Y. Liu, Y. Chen, L.Yuan, H. Liu, J. Wang, Q. Liu, Y. Zhang, Adipose-Derived Stem Cells: Current Applications and Future Directions in the Regeneration of Multiple Tissues, Stem Cells Int, 2020, pp. 8810813, https://doi.org/10.1155/2020/8810813.

[3] Y. Shi, G. Hu, J. Su et al., Mesenchymal Stem Cells: A New Strategy for Immunosuppression and Tissue Repair, Cell Res., Vol. 20, 2010, pp. 510-518, https://doi.org/10.1038/cr.2010.44.

[4] P. A. Zuk, M. Zhu, P. Ashjian, D. A. De Ugarte, J. I. Huang, H. Mizuno, Z. C. Alfonso, J. K. Fraser, P. Benhaim, M. H. Hedrick, Human Adipose Tissue is a Source of Multipotent Stem Cells, Mol. Biol. Cell, Vol. 13, 2002, pp. 4279-4295, https://doi.org/10.1091/mbc.e02-02-0105.

[5] S. Kern, H. Eichler, J. Stoeve, H. Klüter, K. Bieback, Comparative Analysis of Mesenchymal Stem Cells from Bone Marrow, Umbilical Cord Blood, or Adipose Tissue, Stem Cells, Vol. 24, 2006, pp. 1294-1301, https://doi.org/10.1634/stemcells.2005-0342.

[6] Y. Zhu, T. Liu, K. Song, X. Fan, X. Ma, Z. Cui, Adipose-Derived Stem Cell: A Better Stem Cell than BMSC, Cell Biochem. Funct., Vol. 26, 2008, pp. 664-75, https://doi.org/10.1002/cbf.1488.

[7] X. Gu, C. Li, F. Yin, G. Yang, Adipose-Derived Stem Cells in Articular Cartilage Regeneration: Current Concepts and Optimization Strategies, Histol. Histopathol., Vol. 33, 2018, pp. 639-653, https://doi.org/10.14670/HH-11-955.

[8] F. B. Albrecht, T. Ahlfeld, A. Klatt, S. Heine, M. Gelinsky, P. J. Kluger, Biofabrication's Contribution to the Evolution of Cultured Meat, Adv, Healthcare Mater, Vol. 13, 2024, pp. 2304058, https://doi.org/10.1002/adhm.202304058.

[9] P. Kumar, N. Sharma, S. Sharma, N. Mehta, A. K. Verma, S. Chemmalar, A. Q. Sazili, In-vitro Meat: A Promising Solution for Sustainability of Meat Sector, J. Anim. Sci. Technol, Vol. 63, 2021, pp. 693-724, https://doi.org/10.5187/jast.2021.e85.

[10] F. S. Nobre, Cultured Meat and the Sustainable Development Goals, Trends Food Sci. Technol., Vol. 124, 2022, pp. 140-153, https://doi.org/10.1016/j.tifs.2022.04.011.

[11] M. Matsuishi, Science and Technology of Meat and Meat Products in Japan-Pursuit of Their Palatability Under the Influence of Washoku, Traditional Japanese Cuisine, Meat Sci., Vol. 192, 2022, pp. 108919, https://doi.org/10.1016/j.meatsci.2022.108919.

[12] T. Gotoh, T. Nishimura, K. Kuchida, H. Mannen, The Japanese Wagyu Beef Industry: Current Situation and Future Prospects - A Review, Asian-Australas, J. Anim. Sci, Vol. 31, 2018, pp. 933-950, https://doi.org/10.5713/ajas.18.0333.

[13] F. Louis, M. Furuhashi, H. Yoshinuma, S. Takeuchi, M. Matsusaki, Mimicking Wagyu Beef Fat in Cultured Meat: Progress in Edible Bovine Adipose Tissue Production with Controllable Fatty Acid Composition, Mater Today Bio, Vol. 30, 2023, pp. 100720, https://doi.org/10.1016/j.mtbio.2023.100720.

[14] P. Palumbo, F. Lombardi, G. Siragusa, M. G. Cifone, B. Cinque, M. Giuliani, Methods of Isolation, Characterization and Expansion of Human Adipose-Derived Stem Cells (ASCs): An Overview, Int. J. Mol. Sci, Vol. 19, 2018, pp. 1897, https://doi.org/10.3390/ijms19071897.

[15] M. L. B. K. Dominici, K. Le Blanc, I. Mueller et al., Minimal Criteria for Defining Multipotent Mesenchymal Stromal Cells, The International Society for Cellular Therapy Position Statement, Cytotherapy, Vol. 8, 2006, pp. 315-317, https://doi.org/10.1080/14653240600855905.

[16] R. V. Sampaio, M. R. Chiaratti, D. C. N. Santos, F. F. Bressan, J. R. Sangalli, A. L. A. Sa, T. V. Silva, N. N. Costa, M. S. Cordeiro, S. S. Santos, C. E. Ambrosio, Generation of Bovine (*Bos indicus*) and Buffalo (*Bubalus bubalis*) Adipose Tissue Derived Stem Cells: Isolation, Characterization, and Multipotentiality, Genet. Mol. Res., Vol. 14, 2015, pp. 53-62, https://doi.org/10.4238/2015.January.15.7.

[17] Y. Ren, H. Wu, Y. Ma, M. Cang, R. Wang, D. Liu, Isolation, Cultivation and Identification of Adipose-Derived Stem Cell in Bovines, J. Biotechnol., Vol. 26, No. 12, 2010, pp. 1645-1651 (in Chinese).

[18] S. Redaelli, A. Bentivegna, D. Foudah, M. Miloso, J. Redondo, G. Riva, S. Baronchelli, L. Dalprà, G. Tredici, From Cytogenomic to Epigenomic Profiles: Monitoring the Biologic Behavior of *In vitro* Cultured Human Bone Marrow Mesenchymal Stem Cells, Stem Cell Res., Ther, Vol. 3, 2012, pp. 47-47, https://doi.org/10.1186/scrt138.

[19] C. Daniel, Characterization of Bovine Adipose-Derived Stem Cells, Int. J. Sci. Technol. Res., Vol. 6, No. 5, 2017, pp. 16-18, https://doi.org/10.6084/m9.figshare.12602111.

[20] Y. Zhao, S. D. Waldman, L. E. Flynn, The Effect of Serial Passaging on the Proliferation and Differentiation of Bovine Adipose-Derived Stem Cells, Cells Tissues Organs, Vol. 195, No. 5, 2012, pp. 414-427, https://doi.org/10.1159/000329254.

[21] R. Zhu, Y. Feng, R. Li, K. Wei, Y. Ma, Q. Liu, D. Shi, J. Huang, Isolation Methods, Proliferation, and Adipogenic Differentiation of Adipose-Derived Stem Cells from Different Fat Depots in Bovines, Mol. Cell Biochem., Vol. 479, No. 3, 2024. pp. 643-652, https://doi.org/10.1007/s11010-023-04753-9.

[22] M. F. Raoufi, P. Tajik, M. M. Dehghan, F. Eini, A. Barin, Isolation and Differentiation of Mesenchymal Stem Cells from Bovine Umbilical Cord Blood, Reprod. Domest. Anim, Vol. 46, 2011, pp. 95-99, https://doi.org/10.1111/j.1439-0531.2010.01594.x.

[23] S. H. Lee, S. H. Cha, C. L. Kim, H. S. Lillehoj, J. Y. Song, K. W. Lee, Enhanced Adipogenic Differentiation of Bovine Bone Marrow-Derived Mesenchymal Stem Cells, J. Appl. Animal Res., Vol. 43, No. 1, 2014, pp. 15-21, https://doi.org/10.1080/09712119.2014.883320.

[24] C. N. de Moraes, L. Maia, M. C. Dias, C. P. Dell'Aqua, L. S. da Mota, A. Chapwanya, F. D. L. Alvarenga, E. Oba, Bovine Endometrial Cells: A Source of Mesenchymal Stem/Progenitor Cells, Cell Biol Int., Vol. 40, 2016, pp. 1332-1339, https://doi.org/10.1002/cbin.10688.

[25] C. G. Silva, C. F. Martins, E. R. Cunha, H. C. Bessler, T. Cardoso, G. H. L. Martins, S. N. Bao, Isolation, Characterization and Differentiation of Bovine Derived Mesenchymal Stem Cells from Amniotic Fluid, Wharton's Jelly and Adipose Tissue, Anim. Reprod., Vol. 11, 2014, pp. 476.