https://doi.org/10.17221/52/2022-CJAS

Isolation, culture and identification of sheep skeletal muscle satellite cells

*Sailuo Wan[1,2], Qianqian Pan[2,3], Jinbo Wei[2,3], Cuiyun Zhu[2,3], Jing Jing[2,3], Shuaiqi Qin[2,3], Rongcui Hu[2,3], Mengyu Lou[2,3], Shuang Li[2,3], Yinghui Ling[2,3]**

[1]*College of Agricultural Engineering, Anhui Open University, Hefei, P.R. China*
[2]*College of Animal Science and Technology, Anhui Agricultural University, Hefei, P.R. China*
[3]*Anhui Province Key Laboratory of Local Livestock and Poultry Genetic Resource Conservation and Bio-Breeding, Anhui Agricultural University, Hefei, P.R. China*

**Corresponding author: caaslyh@163.com*

Citation: Wan S.L., Pan Q.Q., Wei J.B., Zhu C.Y., Jing J., Qin S.Q., Hu R.C., Lou M.Y., Li S., Ling Y.H. (2022): Isolation, culture and identification of sheep skeletal muscle satellite cells. Czech J. Anim. Sci., 67: 416–423.

Abstract: Skeletal muscle satellite cells (SMSCs) play an important role in muscle growth, regeneration and maintenance. This study aimed to isolate SMSCs from sheep, establish a system for isolation, culture and identification of SMSCs *in vitro*, and provide seed cells for subsequent studies. SMSCs were isolated and purified from newborn 2-day-old healthy sheep by collagenase type I and trypsin two-step digestion and pre-plating method. The results indicated that the isolated and purified SMSCs showed full spindle shape and strong refractive index. The cell growth curve detected by CCK-8 kit was typical "S" type. Immunofluorescence analysis showed that the isolated cells expressed SMSC marker proteins Pax7 and MyoD1. After induction of myogenic differentiation, the cells fused with each other to form multinucleated myotubes and expressed the myoblast specific marker MHC. RT-PCR results showed that the cells expressed SMSC marker gene *Pax 7*. This experiment established an *in vitro* isolation, purification and identification system for sheep skeletal muscle satellite cells, which provided a good cell model for studying the biological mechanism of sheep skeletal muscle cells, optimizing sheep breeds, and cell transplantation repair.

Keywords: SMSC; proliferation; myogenic differentiation; identification

Skeletal muscle is the main component of the fat-free mass of livestock, which is related to the growth and development of the body and meat production (Wang et al. 2021). Usually, the number of muscle fibres is fixed as early as in the foetal period, and the hypertrophy of muscle fibres after birth is mainly caused by the growth of skeletal muscle. Among them, skeletal muscle satellite cells (SMSCs), as a kind of adult stem cells, are closely related to muscle fibre hypertrophy and regeneration.

SMSCs are a type of tissue stem cells with proliferation and differentiation potential located between the muscle cell membrane and basement membrane (Liu et al. 2012). Under normal circumstances, SMSCs are in a relatively resting state, activated when the muscle tissue is stimulated

Supported by the National Natural Science Foundation of China (No. 32172695), the Natural Science Foundation of Anhui Province (No. 2108085Y11), the Anhui Province Academic and Technology Leader Reserve Talent Project (No. 2019H206) and the Support Plan for Outstanding Young Talents in Universities of Anhui Province (No. gxyq2019129).

https://doi.org/10.17221/52/2022-CJAS

or injured by the outside world, and differentiated into new muscle fibres to replace the injured muscle tissue (Relaix and Zammit 2012). Therefore *in vitro* culture of SMSCs is a scientifically proven means when it comes to study the growth and development of muscle cells, to understand the morphological and structural changes of muscle cells and to study the function of muscle growth and development of related genes.

SMSCs were first discovered in frogs (Mauro 1961), since then, different methods have been used to isolate SMSCs from different species (Gharaibeh et al. 2008; Kamanga-Sollo et al. 2011; Montoya-Flores et al. 2012). Reported to be firstly isolated from rat, SMSCs are now diversely isolated and furthermore cultured *in vitro*, from different species like humans, chicken, sheep, cattle and pigs (Minnaard et al. 2009; Lei et al. 2011; Yan et al. 2013; Li et al. 2014; Li et al. 2015). Studies have shown that paired box 7 (*PAX7*) is a specific marker gene for quiescent and growth phases of skeletal muscle cells, and it is often used to identify whether isolated cells are SMSCs (Schmidt et al. 2019). Furthermore, the myogenic regulatory factor, myogenic differentiation 1 (MyoD1), is a marker of the proliferation and differentiation of satellite cells (Buckingham et al. 1992). Desmin is abundantly expressed in skeletal muscle cells and can be used to identify skeletal muscle cells in other tissues. Myosin heavy chain (MHC) protein is the most common marker molecule for muscle fibre types (Sawano and Mizunoya 2022). Therefore, these four proteins can be used to more comprehensively characterize SMSCs. SMSCs for their scientific value either from theoretical or practical view play a crucial role for improving livestock and poultry meat production. This study established an isolated and identifiable system of SMSCs, which is expected to provide an ideal model for regulating sheep muscle growth and development at the molecular level.

MATERIAL AND METHODS

Ethics statement

Animal protocols were approved by the Review Committee for the Use of Animal Subjects of Anhui Agricultural University. The sheep were euthanized following the intravenous injection of a barbiturate (30 mg/kg).

Sheep muscle tissue collection

Sheep used in the experiment were provided by Lujiang Xiangrui Breeding Co., Ltd. (Hefei, China). The *longissimus dorsi* muscle was collected from 2-day-old sheep. The collected muscle tissue was thoroughly disinfected with iodophor and 75% alcohol. After the muscle tissue was removed from alcohol, it was placed in phosphate buffer solution (PBS) (HyClone, USA) containing penicillin-streptomycin liquid (PS; Solarbio, Beijing, China).

Isolation and culture of sheep skeletal muscle satellite cells

The *longissimus dorsi* muscle tissue was removed and placed in a 100 mm dish, and the surface tissue was resected again with forceps and ophthalmic scissors. Then, the tissue was moved to a new dish containing PBS buffer solution with 5% of PS and the skin, blood vessels, adipose tissue and connective tissue were removed. Next, muscle tissue was reduced into paste. Collagenase type I (0.1% Solarbio, Beijing, China) was added for a further enzymatic digestion at 37 °C for 1 h and was agitated every 10 min. After centrifugation at 1 000 r/min for 10 min, the supernatant was discarded and 0.25% trypsin (HyClone, Logan, UT, USA) was added, which was digested at 37 °C for 30 min and shaken every 10 minutes. DMEM/F12 (HyClone, Logan, UT, USA) medium containing 20% foetal bovine serum (FBS) (Cat. No. 10099-141; Gibco, Grand Island, NY, USA) was added to suspend the second enzymatic digestion. Cell suspensions were respectively filtered through 100 µm, 200 µm and 400 µm mesh cell screens. The filtered cell suspensions were centrifuged at 1 000 r/min for 10 min, and the supernatant was discarded. The cells were suspended in DMEM/F12 medium containing 20% FBS, seeded onto 100 mm petri dish and were cultured in a 5% CO_2 incubator at 37 °C for 1 hour. The non-adherent cell suspensions were transferred to a new culture plate for the second adherence to remove fibroblasts.

Growth curve drawing

SMSCs cells at the logarithmic growth stage of the third subcultured generation were digested and collected, and inoculated into 96-well plates

https://doi.org/10.17221/52/2022-CJAS

at a concentration of 1 × 10^4 cells/ml. They were divided into seven groups with six replicates per group. CCK-8 (Solarbio, Beijing, China) was added (10 µl) to each column of wells in turn every day, and they were incubated for 3 h, and the absorbance value at 450 nm was measured with a microplate reader. Results were used to draw the growth curve by GraphPad Prism 8 (GraphPad Software, Inc., San Diego, CA, USA).

Immunofluorescence staining

SMSCs at the third subcultured generation were digested and collected and inoculated into 12-well plates with 5 × 10^4 cells/ml. When the cells reached 50% to 60% of confluency, PBS buffer was used to wash them in order to proceed to the immunofluorescence assay. The cells were fixed with 4% paraformaldehyde (Biosharp, Shanghai, China) precooled at 4 °C for 20 min and rinsed with PBS buffer three times. The cells were permeabilized with 0.25% Triton X-100 (Solarbio, Beijing, China) per well for 10 min and rinsed with PBS buffer three times. 3% bovine serum albumin (BSA) (Sigma-Aldrich, St. Louis, MO, USA) was added and sealed at room temperature for 2 hours. The sealing solution was removed, the blocking solution was added with primary antibodies of Pax7 (Santa Cruz Biotechnology, Dallas, TX, USA) and MyoD1 (Santa Cruz Biotechnology, Dallas, TX, USA) at 1 : 200 dilutions, and incubated overnight at 4 °C. After three washes for 5 min each time in PBS, goat anti-rat 594 labelled secondary antibody (Jackson ImmunoResearch, West Grove, PA, USA) was added and incubated at 37 °C for 1 h, and followed by washing three times in PBS again. Cell nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI, Beyotime Biotech, Jiangsu, China) for 15 minutes. The plates were sealed and observed under a fluorescence microscope.

Myogenic differentiation and immunofluorescence staining

The cells were cultured to the third generation, and when the confluency reached 70%, the culture medium was discarded and the cells were washed with PBS buffer solution. The culture medium (DMEM-F12 culture medium containing 2% FBS) was used to induce differentiation for seven days, and the differentiation of SMSC was observed under a microscope. The specific steps of MHC (R&D Systems, Minneapolis, MN, USA) and desmin (Cell Signaling Technology, Danvers, MA, USA) expression detection by cellular immunofluorescence are described in the above section.

SMSC were identified by RT-RCR

TRIzol was used to lysate the third-generation SMSC and extract the total RNA of the cells, which was reversed into cDNA according to the instructions of the kit (Aidlab, Beijing, China). Expression of the specific marker gene PAX7 was detected by RT-PCR. All primers were synthesized by Shenggong Bioengineering (Shanghai, China) Co., Ltd. The PCR reaction system consisted of: 12.5 µl of 2×Taq PCR Master Mix, 0.5 µl of primer pairs (Table 1), 2 µl of cDNA, 9.5 µl of ddH_2O. PCR reaction conditions were: pre-denaturation for 5 min at 94 °C; 94 °C denaturation 30 s, annealing temperature 58 °C for 30 s, 72 °C extension 1 min, a total of 35 cycles; extension at 72 °C for 10 minutes. Amplification products were detected by 2% agarose gel electrophoresis.

RESULTS

Morphological observation of sheep skeletal muscle satellite cells

The newly isolated sheep skeletal muscle satellite cells adhered to the plate and obviously started to proliferate. After 72 h, most of the cells adhered to the wall and grew, and the cells were full, spindle-shaped, and had strong refraction. When the satellite cell confluency reached 80%, they can be subcultured or cryopreserved. However, the obtained cells contained a certain amount of fibroblasts at this time, the cell suspen-

Table 1. Primers for real-time polymerase chain reaction

Gene name	Primer sequence (5'–3')	Size (bp)
GAPDH	F:CACAGTCAAGGCAGAGAAC R:TACTCAGCACCAGCATCA	108
Pax7	F:CGACCCCTGCCTAATCACATCCGC R:GAGTCGCCACCTGTCTGGGCTTGC	190

https://doi.org/10.17221/52/2022-CJAS

sion was transferred to a new culture dish after 1 h during subsequent subpassage and resuscitation. Purified to the third generation of spindle-shapes cells, refraction is strong, they are of uniform shape (Figure 1). When the cell confluency reached 80%, the medium was replaced with myogenic differentiation medium, and the cells were gradually elongated and thinned to form the regular parallel arrangement. Further culture showed the more obvious directional arrangement of cells that could spontaneously fuse to form myotubes. At this time, several to dozens of cell nuclei were arranged in the centre of the cell like beads (Figure 1). The results indicated that the isolated satellite cells of sheep muscle had good differentiation ability *in vitro*.

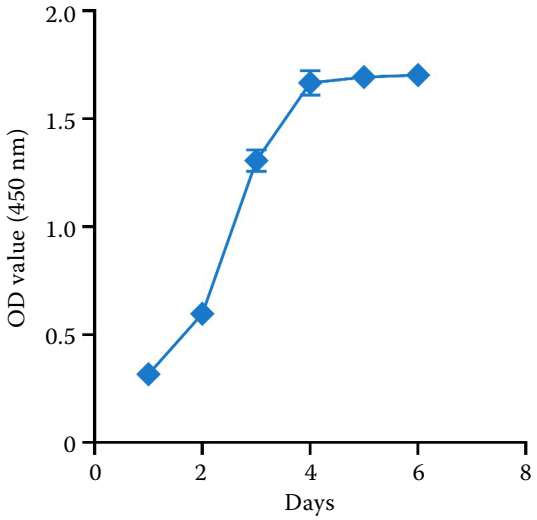

Figure 2. The skeletal muscle satellite cell growth curve

Growth curves of sheep skeletal muscle satellite cells

In order to determine the growth rate of SMSC in *in-vitro* culture, isolated SMSC cells were inoculated in the medium containing 20% FBS for subculture, and the growth curve of the third generation cells at the logarithmic growth stage was measured and plotted. As shown in Figure 2, the growth curve of the third generation of SMSCs was "S" type, with incubation period of 1–2 days and logarithmic growth period of 2–4 days. The number of cells reached the maximum on the 4th day.

Immunofluorescence identification of sheep skeletal muscle satellite cells

Immunofluorescence was used to detect the expression of Pax7 and MyoD1 in SMSC. Immunofluorescence results at the proliferation stage of SMSCs showed that Pax7 and MyoD1 antibodies combined well with the proteins in the cells, which was consistent with the characteristics of skeletal muscle satellite cells (Figure 3).

In this study, SMSC myoblast differentiation was induced by a serum withdrawal method for seven days. Myotube formation was observed under a microscope, and they gradually thickened and matured with the extension of differentiation time. The expression of MHC and desmin, the specific markers of myoblast differentiation, was detected by immunofluorescence assay. The results showed that MHC and desmin showed positive expression in the muscle tubes after differentiation induction, indicating that the separated SMSCs had the ability to fuse into myotubes (Figure 4).

SMSCs myoblast differentiation was induced by a serum withdrawal method for seven days. Myotube formation can be clearly observed under

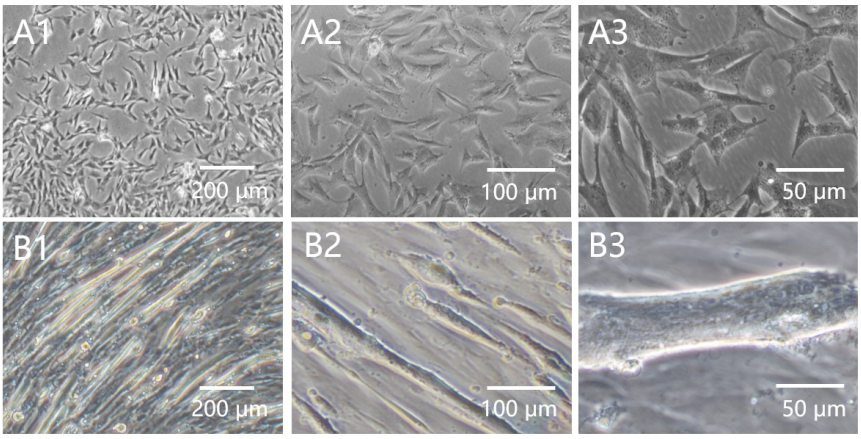

Figure 1. Skeletal muscle satellite cell morphology
Cell morphology of A1, A2 and A3 was observed under a white light microscope after three days of cell proliferation. Cell morphology of B1, B2 and B3 was observed under a white light microscope in seven days after cell differentiation

https://doi.org/10.17221/52/2022-CJAS

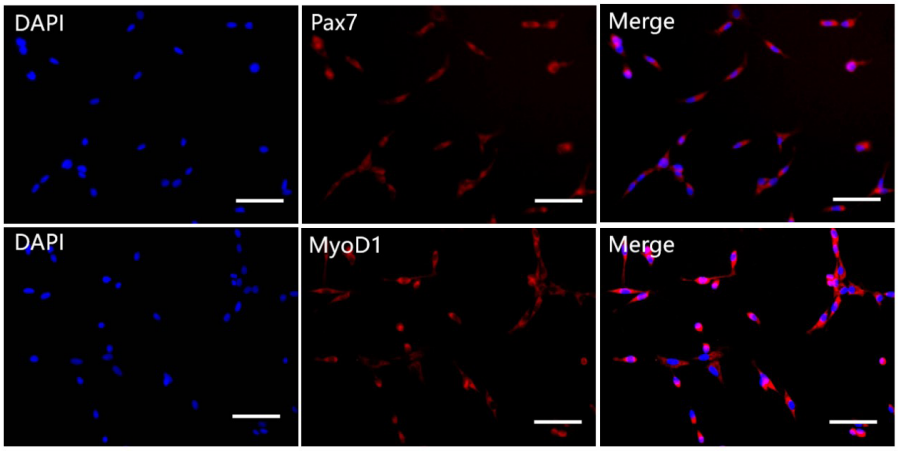

Figure 3. Immunofluorescence identification of skeletal muscle satellite cell Pax7 and MyoD1
Scale bar: 50 um

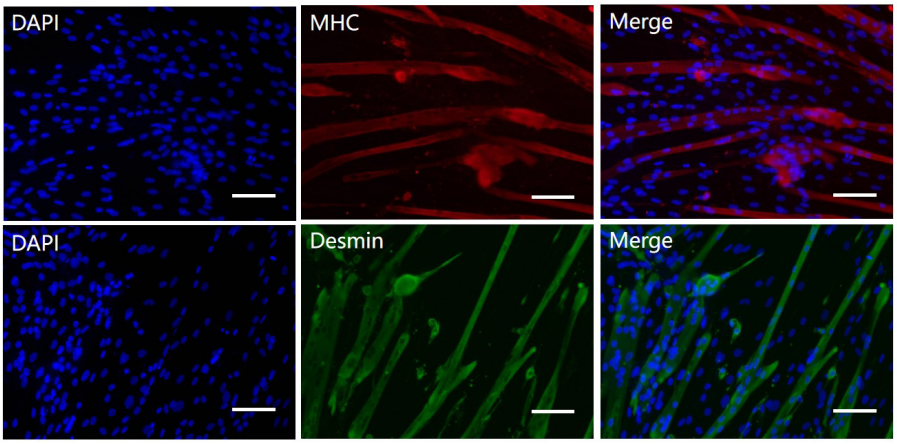

Figure 4. Immunofluorescence identification of myosin heavy chain (MHC) and desmin on day 7 of myoblast-induced differentiation of skeletal muscle satellite cells
Scale bar: 50 um

a microscope. After MHC immunofluorescence staining, the myotube morphology was more obvious. It can be clearly observed that myotubes are formed in a large proportion (Figure 5). This indicates that the skeletal muscle satellite cells isolated in this experiment are in good condition and suitable for this experimental culture system.

Identification of sheep skeletal muscle satellite cells by RT-PCR

The total RNA extracted from SMSCs was detected by UV spectrophotometer. The ratio of 260 nm/A280 nm ranged between 1.9 and 2.1, indicating that the RNA quality was good and could be used for subsequent RT-PCR identification. The amplified fragments were 190 bp, and the band sizes were consistent with expectations, indicating that the isolated cells express *Pax7*, which is in line with the typical characteristics of SMSCs (Figure 6).

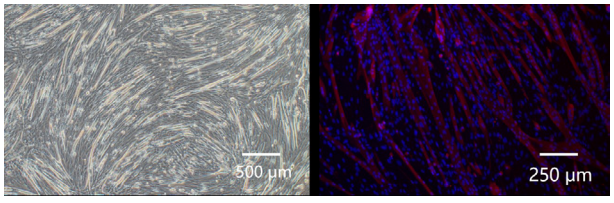

Figure 5. The morphology and number of myotubes induced by skeletal muscle satellite cells

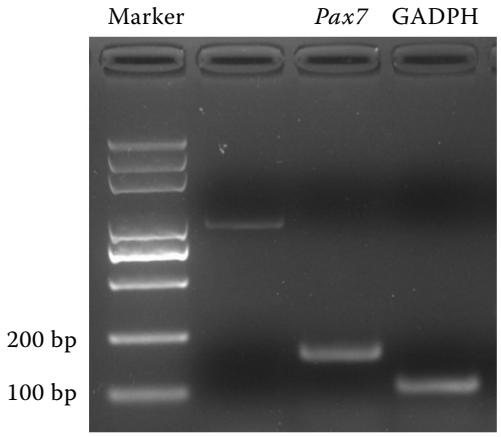

Figure 6. Electrophoresis of the PCR product of *Pax7* genes

https://doi.org/10.17221/52/2022-CJAS

DISCUSSION

Isolation and culture of SMSCs

SMSCs are a commonly used model to study the mechanisms of muscle growth and development. Sheep belong to the important economic animals and have made great contributions to meet the nutritional needs of human beings. Therefore, it is of great significance to use the sheep skeletal muscle satellite cell model to study the growth and development of sheep skeletal muscle.

A previous study reported that sheep SMSCs were isolated and purified using two-step enzymatic digestion methods (Wu et al. 2012). In contrast, in this study, the *longissimus dorsi* muscle of 2-day-old sheep was selected as the sample for isolating SMSCs rather than the leg muscle tissue of 90-day-old foetus of sheep. SMSCs in animals are a kind of adult stem cells, and their content is related to age. Studies have shown that the number of SMSCs decreased as the age increased. In adult animals, muscle satellite cells accounted for only 1% to 5% (Renault et al. 2002; Forcina et al. 2019). Therefore, in this study, sheep aged two days were selected for the isolation of SMSCs. Currently, there are two main methods for isolating skeletal muscle satellite cells. The first method is to release SMSCs by breaking down the connective tissue network and muscle fibres through mincing, enzymatic digestion and repeated grinding of muscle mass (Kamanga-Sollo et al. 2011; Montoya-Flores et al. 2012; Wang et al. 2020). This is a classic and efficient method to obtain sufficient muscle satellite cells, although this method can obtain a heterogeneous population of precursor cells. The second method is to isolate the SMSCs from a single intact muscle fibre, when relatively pure SMSCs can be obtained (Kastner et al. 2000; Keire et al. 2013). Besides, flow separation method and density gradient centrifugation method are also used for separation and purification for isolating SMSCs (Wu et al. 2018). Flow cytometry, mainly used in human and mouse studies, makes it possible to separate cells with a purity of 99%, but the equipment is expensive and prone to contamination (Maesner et al. 2016). Although the density gradient centrifugation method can obtain high purity cells, it can cause a large loss of the cell number and has a certain influence on cell activity due to repeated centrifugation. Therefore, in this study, the pre-plating method published by Richler and Yaffe was used to purify skeletal muscle satellite cells (Richler and Yaffe 1970). The pre-plating method is based on the fact that fibroblasts can adhere to the wall in 0.5 to 1 hour. The cell suspension is transferred to a new culture dish every 1 h, which greatly reduces the time and workload of purifying cells, and the purification effect is improved. The two-step digestion method can fully release the cells, and the differential adhesion method can remove the fibroblasts. The obtained SMSCs have high purity and strong proliferation and differentiation ability after passage. In summary, in this study, sheep SMSCs were isolated by a two-step digestion method of 0.1% collagenase type I and 0.25% trypsin. In this study, sheep SMSCs were isolated and cultured in 20% FBS medium, and further purified by the pre-plating method.

Differentiation and identification of SMSCs

In this study, 20% FBS was used to promote the proliferation of sheep SMSCs, and 2% FBS was used after the cell convergence reached 80% to induce myogenic differentiation and the formation of myotubes. In addition to morphological changes, the synthesis and expression of some specific proteins of SMSCs are also important markers of differentiation, which can be used as indicators of identification of SMSCs. At present, specific markers of the resting phase of SMSCs include muscle nuclear factor (MNF), hepatocyte growth factor receptor (C-MET), Pax7, and M-cadherin. Specific marker proteins at the proliferation stage include Myf5, MyoD1 and desmin, and specific marker protein MHC at the differentiation stage (Zhang et al. 2020; Sincennes et al. 2021). MHC plays an important role in skeletal muscle development and is often used as marker genes for muscle formation. In this experiment, Pax7 and MyoD1 specific cell marker proteins were used for fluorescence identification of cells in the proliferation stage, and the results showed positive expression. MHC and desmin specific cell marker proteins were used for fluorescence identification of cells in the differentiation stage, and the results showed positive expression and obvious myotube of MHC and desmin. It was preliminarily confirmed that the isolated cells were sheep SMSCs with differentiation potential.

https://doi.org/10.17221/52/2022-CJAS

In this study, sheep SMSCs were successfully isolated and identified by immunofluorescence detection of the marker protein of SMSCs. A method for the isolation, purification and identification of sheep SMSCs *in vitro* was established, and muscle-induced differentiation was successfully achieved, which provided experimental materials and technical support for future studies on the molecular mechanism of the regulation of sheep skeletal muscle growth and development

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

Buckingham ME, Lyons GE, Ott MO, Sassoon DA. Myogenesis in the mouse. In: Chadwick DJ, Marsh J, editors. Postimplantation development in the mouse: Postimplantation development in the mouse. Ciba Foundation Symposium 165. Chichester: John Wiley and Sons; 1992. p. 111-31.

Forcina L, Miano C, Pelosi L, Musaro A. An overview about the biology of skeletal muscle satellite cells. Curr Genomics. 2019 Jan 1;20(1):24-37.

Gharaibeh B, Lu A, Tebbets J, Zheng B, Feduska J, Crisan M, Peault B, Cummins J, Huard J. Isolation of a slowly adhering cell fraction containing stem cells from murine skeletal muscle by the preplate technique. Nat Protoc. 2008 Sep;3(9):1501-9.

Kamanga-Sollo E, White ME, Hathaway MR, Weber WJ, Dayton WR. Effect of trenbolone acetate on protein synthesis and degradation rates in fused bovine satellite cell cultures. Domest Anim Endocrinol. 2011 Jan;40(1):60-6.

Kastner S, Elias MC, Rivera AJ, Yablonka-Reuveni Z. Gene expression patterns of the fibroblast growth factors and their receptors during myogenesis of rat satellite cells. J Histochem Cytochem. 2000 Aug;48(8):1079-96.

Keire P, Shearer A, Shefer G, Yablonka-Reuveni Z. Isolation and culture of skeletal muscle myofibers as a means to analyze satellite cells. Methods Mol Biol. 2013 Apr 23; 946:431-68.

Lei HL, Yu B, Yang XR, Liu ZH, Huang ZQ, Mao XB, Tian G, He J, Han GQ, Chen H, Mao Q, Chen DW. Inhibition of adipogenic differentiation by myostatin is alleviated by arginine supplementation in porcine-muscle-derived mesenchymal stem cells. Sci China Life Sci. 2011 Oct; 54(10):908-16.

Li YH, Li FN, Lin BB, Kong XF, Tang YL, Yin YL. Myokine IL-15 regulates the crosstalk of co-cultured porcine skeletal muscle satellite cells and preadipocytes. Mol Biol Rep. 2014 Nov;41(11):7543-53.

Li BJ, Li PH, Huang RH, Sun WX, Wang H, Li QF, Chen J, Wu WJ, Liu HL. Isolation, culture and identification of porcine skeletal muscle satellite cells. Asian-Australas J Anim Sci. 2015 Aug;28(8):1171-7.

Liu Y, Chen S, Li W, Du H, Zhu W. Isolation and characterization of primary skeletal muscle satellite cells from rats. Toxicol Mech Method. 2012 Nov;22(9):721-5.

Maesner CC, Almada AE, Wagers AJ. Established cell surface markers efficiently isolate highly overlapping populations of skeletal muscle satellite cells by fluorescence-activated cell sorting. Skelet Muscle. 2016 Dec; 6(1): 10 p.

Mauro A. Satellite cell of skeletal muscle fibers. J Biophys Biochem Cytol. 1961 Feb;9(2):493-5.

Minnaard R, Schrauwen P, Schaart G, Jorgensen JA, Lenaers E, Mensink M, Hesselink MKC. Adipocyte differentiation-related protein and OXPAT in rat and human skeletal muscle: Involvement in lipid accumulation and type 2 diabetes mellitus. J Clin Endocr Metab. 2009 Oct; 94(10):4077-85.

Montoya-Flores D, Mora O, Tamariz E, Gonzalez-Davalos L, Gonzalez-Gallardo A, Antaramian A, Shimada A, Varela-Echavarria A, Romano-Munoz JL. Ghrelin stimulates myogenic differentiation in a mouse muscle satellite cell line and in primary cultures of bovine myoblasts. J Anim Physiol Anim Nutr. 2012 Aug;96(4):725-38.

Relaix F, Zammit PS. Satellite cells are essential for skeletal muscle regeneration: The cell on the edge returns centre stage. Development. 2012 Aug;139(16):2845-56.

Renault V, Rolland E, Thornell LE, Mouly V, Butler-Browne G. Distribution of satellite cells in the human vastus lateralis muscle during aging. Exp Gerontol. 2002 Dec;37(12):1513-4.

Richler C, Yaffe D. The in vitro cultivation and differentiation capacities of myogenic cell lines. Dev Biol. 1970 Sep; 23(1):1-22.

Sawano S, Mizunoya W. History and development of staining methods for skeletal muscle fiber types. Histol Histopathol. 2022 Jun;37(6):493-503.

Schmidt M, Schuler SC, Huttner SS, Von Eyss B, Von Maltzahn J. Adult stem cells at work: Regenerating skeletal muscle. Cell Mol Life Sci. 2019 Jul;76(13):2559-70.

Sincennes MC, Brun CE, Lin AYT, Rosembert T, Datzkiw D, Saber J, Ming H, Kawabe YI, Rudnicki MA. Acetylation of PAX7 controls muscle stem cell self-renewal and differentiation potential in mice. Nat Commun. 2021 May 31;12(1): 15 p.

https://doi.org/10.17221/52/2022-CJAS

Wang Y, Xiao X, Wang L. In vitro characterization of goat skeletal muscle satellite cells. Anim Biotechnol. 2020 Apr;31(2):115-21.

Wang H, He K, Zeng XH, Zhou XL, Yan FF, Yang SB, Zhao A. Isolation and identification of goose skeletal muscle satellite cells and preliminary study on the function of C1q and tumor necrosis factor-related protein 3 gene. Anim Biosci. 2021 Jun;34(6):1078-87.

Wu H, Ren Y, Li S, Wang W, Yuan J, Guo X, Liu D, Cang M. In vitro culture and induced differentiation of sheep skeletal muscle satellite cells. Cell Biol Int. 2012 Jun 1; 36(6):579-87.

Wu J, Matthias N, Lo J, Ortiz-Vitali JL, Shieh AW, Wang SH, Darabi R. A myogenic double-reporter human pluripotent stem cell line allows prospective isolation of skeletal muscle progenitors. Cell Rep. 2018 Nov 13;25(7):1966-81.

Yan J, Gan L, Yang HL, Sun C. The proliferation and differentiation characteristics of co-cultured porcine preadipocytes and muscle satellite cells in vitro. Mol Biol Rep. 2013 Apr;40(4):3197-202.

Zhang H, Wen J, Bigot A, Chen J, Shang R, Mouly V, Bi P. Human myotube formation is determined by MyoD-Myomixer/Myomaker axis. Sci Adv. 2020 Dec;6(51): 14 p.

Received: March 14, 2022
Accepted: October 7, 2022
Published online: October 20, 2022