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Effect of Sex Steroid Hormones on Bovine Myogenic Satellite Cell Proliferation, Differentiation and Lipid Accumulation in Myotube*

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ABSTRACT : Myogenic satellite cells (MSCs) are adult stem cells that activate and differentiate into myotubes. These stem cells are multipotent as they transdifferentiate into adipocyte-like cells, nerve cells and osteocytes. The effects of steroid hormones (E_2 and testosterone) were studied as a further step toward understanding the mechanism of MSCs proliferation and differentiation. In this study, MSCs were grown continuously for 87 days, implying that there may be a group of MSCs that continue to proliferate rather than undergoing differentiation. Isolated MSCs were cultured in Dulbecco's Modified Eagle's Medium supplemented with adult male, female or castrated bovine serum to observe the effect of steroid hormones on MSC proliferation. Cell proliferation was the highest in cultures supplemented with male serum followed by female and castrated serum. The positive effect of male hormone on MSC proliferation was confirmed by the observation of testosterone-mediated increased proliferation of cells cultured in medium supplemented with castrated serum. Furthermore, steroid hormone treatment of MSCs increased lipid accumulation in myotubes. Oil-Red-O staining showed that 17β-estradiol (E_2) treatment avidly increased lipid accumulation, followed by E_2+testosterone and testosterone alone. To our knowledge, this is the first report of lipid accumulation in myotubes due to steroids in the absence of an adipogenic environment, and the effect of steroid hormones on cell proliferation using different types of adult bovine serum, a natural hormonal system. In conclusion, we found that sex steroids affect MSCs proliferation and differentiation, and lipid accumulation in myotubes. (**Key Words** : Myogenic Satellite Cells, Steroids, Serum, Stem Cells, Bovine)

INTRODUCTION

Myogenic satellite cells (MSCs) are a pool of undifferentiated and mononucleated myogenic precursors located beneath the basal lamina of myofibers. MSCs are able to fuse with closely located myotubes or with the other MSCs to form new myotubes (Chen and Goldmer, 2003). Thus, they represent a stem cell population responsible for the post-natal growth and muscle repair. MSCs exist in a quiescent state after birth and begin to proliferate in response to regulatory factors during development and in cases of muscle injury (Allen and Rankin, 1990; Allen et al., 1997; Cornelison and Wold, 1997). MSCs possess the multipotential capacity to form adipocyte-like cells, osteocytes in addition to myocytes (Asakura et al., 2001; Fux et al., 2004). Involvement of several transcriptional factors has been described in the differentiation of MSCs into myotubes. The MyoD family of bHLH transcription factors is required for the commitment and differentiation of embryonic myoblasts during development. Increased MyoD, myogenin and other myogenic regulatory factors (MRFs) initiates the terminal myogenic differentiation program by upregulating genes encoding creatine kinase, myosin heavy chain and acetylcholine receptor subunits that underlie the functional properties of mature muscle (Hawke and Garry, 2001). MRFs such as myogenin, MRF4, MyoD and Myf5 are expressed in anatomical- and time-dependent manners (Buckingham et al., 2003).

Adipocyte differentiation begins during development; adipose tissue is formed in various parts of the body before birth with intramuscular adipose tissue formed at last.

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Myoblasts and adipocytes both arise from the mesoderm layer of the embryo. Fatty acids and glucose constitute the primary oxidative fuels that support skeletal muscle contractile activity. In the context of normal physiology, this tissue harbors great potential to adjust fuel selection to match both energy supply and demand. However, abnormal lipid droplets within non-adipose tissues including skeletal muscle is the central component of obesity, type 2 diabetes and cardiovascular disease, which also leads to insulin resistance (Goodpaster and Kelley, 1998; Hulver et al., 2003). Concerning meat consumption, beef with high marbling is valued for its increased tenderness, juiciness and flavor intensity (Wheeler et al., 1994; Van Barneveld, 2003). Therefore, enhancement of the intramuscular adipose tissue has been the subject of interest among several researchers.

Endocrine factors influence muscle growth and development throughout life, and the extremes of hormonal excess or deficiency adversely affect muscle structure and function (Veldhuis et al., 2005). Skeletal muscle is a target tissue for estrogen, which exerts an anti-apoptotic action (Boland et al., 2008) by blocking the apoptotic morphological changes of cell organelles such as nucleus, mitochondria and also preventing cytochrome c release, caspase-3, PARP cleavage and DNA fragmentation (Vasconsuelo et al., 2008). Moreover, androgen and estrogen receptors have been detected in skeletal muscle (Snochowski et al., 1981; Meyer and Rapp, 1985), indicating a possible mode of action for sex steroids as direct stimulating agents of the muscle. In addition, testosterone increases muscle mass and strength by inducing hypertrophy of muscle fibres, and increasing myonucleus and MSC numbers (Mooradian et al., 1987; Dehm and Tindall, 2007). Previously, we demonstrated that steroid hormones are crucial in maintaining the normal morphology and growth of MSCs (Lee et al., 2007). The role of insulin-like growth factor-1 (IGF-1) in 17β-estradiol (E_2) and androgen-stimulated MSC proliferation has been studied (Kamanga-Sollo et al., 2008b).

The present study was undertaken to determine the effect of steroid hormones on MSCs proliferation, differentiation and lipid accumulation in myotube. We report that male steroid hormone plays an important role in MSC proliferation, whereas female steroid hormone is influential in lipid accumulation in myotubes. Meat quality may be defined as the meat with marbling. Therefore, increase in myogenic satellite cell proliferation and differentiation will increase the meat quantity, and increase in lipid accumulation will increase marbling. Thus, the study will enhance the understanding of the mechanism of MSC differentiation and lipid accumulation in differentiated myotubes, which may ultimately help in the production of meat quantity and quality in the near future.

MATERIALS AND METHODS

Sample collection

Bovine hind limb skeletal muscles and blood for serum extraction were collected in airtight plastic bags and separate sterilized bottles, respectively, from a regional slaughter-house. The slaughtered cattle ranged in age from 24-26 months and in body weight from 550-600 kg.

MSC culture

Myogenic satellite cells were isolated and cultured according to Garriga et al. (2000) and Lee et al. (2007) with slight modifications. Skeletal muscle was washed in phosphate buffered saline (PBS), minced into fine pieces using sterilized scissors and digested by trypsin-EDTA (GIBCO, Carlsbad, CA, USA) for 2 h. Digested tissue was centrifuged at 90 g for 3 min and the upper phase was filtered using a 40 μm diameter pore size cell strainer. The filtrate was centrifuged at 2,500 rpm at room temperature for 20 min. The digestion medium was removed leaving the cell pellet in the tube. The collected cell pellet was washed three times by Dulbecco's Modified Eagle's Medium (DMEM; HyClone Laboratories, Logan, UT, USA) containing 1% penicillin/streptomycin (Invitrogen, CA, USA) and cultured in a 100 mm-diameter culture dish using DMEM supplemented with 10% fetal bovine serum (FBS; HyClone Laboratories), 1% penicillin/streptomycin, 0.1% amphotericine (GIBCO) by incubating at 37°C in an atmosphere of 5% CO_2. The condition of each primary MSC culture was checked every day and the medium was changed every second day. To ascertain the effect of different adult serum, the primary MSC culture was subcultured to passage 1 when the culture reached confluence. The cultured cells were harvested by trypsin-EDTA and subcultured in 6-well plates after washing the collected cell pellet with DMEM supplemented with 1% penicillin/streptomycin. During subculture, cells were incubated in DMEM supplemented with 2% or 10% adult bovine serum to observe MSC proliferation.

Immunocytochemistry

Isolated MSCs were cultured in a cover glass-bottom dish. For myogenin staining, cells were allowed to differentiate into myotubes prior to removal of the medium and rinsing with PBS. PBS rinsing was also done between all subsequent steps. Cells were fixed with 4% formaldehyde and excess fixative was removed by a PBS wash. Cells were permeablized by 0.2% Triton X-100 and 3-4 drops of Image-iT^{TM} FX signal enhancer (Invitrogen) was applied. Primary antibody (1:50; antibody: PBS, mouse

monoclonal IgG_1 Myogenin; sc-12732; Santa Cruz Biotechnology, Santa Cruz CA, USA) was added and incubated at 4°C in humid environment overnight and then incubated with secondary antibody (Alexa Fluor 488 Goat Anti-mouse SFx kit; Molecular Probes, Eugene, OR, USA) at room temperature for 1 h. 4' 6'-diamino-2-phenylindole (DAPI; Sigma-Aldrich, St. Louis MO, USA) was added for nuclear staining. Immunostained cells were observed using a fluorescence microscope (Nikon, Tokyo, Japan).

Serum extraction

Blood from male, female and castrated adult bovines was collected and transported to the laboratory at 4°C. To avoid coagulation during transport, 300 units of heparin sodium salt from bovine intestinal mucosa (Sigma-Aldrich) was added per 5 ml blood. At the laboratory, each blood sample was sterilized by incubation at 55°C, centrifuged at 11,000 rpm, 4°C, 30 min and the supernatant was collected. The process was repeated two more times. The final obtained serum was stored at -80°C until used. The serum was centrifuged at 3,000 rpm, 4°C, 10 min and filtered through a 0.2 μm diameter pore size filter prior to use.

Hormone treatment

Cells were cultured in steroid-free, charcoal-dextran treated FBS (CDFBS, charcoal-dextran treatment removes steroid components) to observe the steroidal effect on the cultured muscle cells. E_2 only, testosterone only and a combination of E_2+testosterone (all 10 nM) were added directly to DMEM without phenol-red (Fresh Media™, WelGENE, Daegu, South Korea) supplemented with 10% CDFBS. Cells were cultured in steroid-free media with addition of hormones for 2 weeks prior to Oil-Red-O staining to observe lipid accumulation. CDFBS was prepared according to previously described method. The charcoal-dextran mixture was incubated in a 56°C water bath for 45 min with shaking at 6 min intervals and centrifuged two times with a change to a fresh tube between centrifugations and with repeated filtration (Choi et al., 2000). Similarly, to observe the effect of steroid hormones on MSC proliferation, cells subcultured in DMEM supplemented with bovine castrated serum were exposed to hormones (E_2, testosterone and E_2+testosterone) for 7 days.

Cell counting

To study cell proliferation under the influence of different adult bovine serum, MSCs were subcultured in culture media supplemented with male, female and castrated serum and number of cells per well were enumerated. Pictures of three randomly selected regions of each culture well were taken during daily microscopic observation, and the cells present in each picture were counted. The number of cells under three randomly selected area under the microscope were added and considered as the cell numbers per well.

Oil-Red-O staining

A stock solution of Oil-Red-O (Sigma-Aldrich) was prepared by mixing 5 mg/ml of Oil-Red-O in 60% tri-ethyl phosphate (Sigma-Aldrich). The stock solution was diluted (12:8) in autoclaved de-ionized water to produce the working Oil-Red-O solution. The working solution was filtered through Whatman 110 mm paper (Whatman International, Maidstone, UK) before use. Cells cultured in 6-well plates were fixed by 10% formalin for 10 min after removing the culture media. The formalin fixed cells were washed three times using autoclaved de-ionized water. The working solutions of Oil-Red-O (1 ml/well) were added to the wells and 15 minutes later the staining solution was removed, and each well was washed using autoclaved de-ionized water. After air-drying, deionized water was added to each well prior to microscopy.

RNA extraction

Trizol™ reagent (Invitrogen) was used to extract total RNA from cells, according to the manufacturer's protocol. RNA was stored in diethylpyrocarbonate-treated water at -80°C until use. Concentrations of the extracted RNA samples were measured using an ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) and the purity was checked with an Agilent 2100 bioanalyzer (Agilent Technologies, Palo Alto, CA, USA) prior to reverse transcription-polymerase chain reaction (RT-PCR).

Real time RT-PCR

RNA was reverse-transcribed into the first strand cDNA using Superscript-II reverse transcriptase (Invitrogen). Total RNA (1 μg in 20 μl total volume) was primed with oligo (dT) 20 primers (Bioneer, Daejeon, Korea), and reverse transcription was carried out at 42°C for 50 min and 72°C for 15 min. Subsequently, 2 μl of the 10× diluted cDNA product and 10 pmoles of each gene-specific primer were used to perform PCR using a 7500 real time PCR system (Applied Biosystems, Foster City, CA, USA). Power SYBR® Green PCR Master Mix (Applied Biosystems, Warrington, UK) was used as the fluorescence source. Primers were designed with Primer 3 software (http://frodo.wi.mit.edu) using sequence information listed at the National Center for Biotechnology Information. Primers information for glyceraldehyde 3-phosphate dehydrogenase (GAPDH), estrogen receptor-alpha (ER-α), and androgen receptor (AR) was given in our previous paper (Lee et al., 2007). The following are the gene sequences for primers: (progesterone receptor) PR; 5'-

aaatgaacagcggatgaagg-3' and 5'- ctccttttgcctcaaacca-3' (52°C), Desmin; 5'-tgtcgaaaccagacctcaca-3' and 5'-gtggcggtactccatcatct-3' (57°C), and Myogenin; 5'-tgggcgtgtaaggtgtgtaa-3' and 5'- tgcaggcgctctatgtactg-3' (57°C). RT-PCR was carried out under the following conditions: pre-denaturation of the synthesized cDNA at 95°C for 10 min was followed by 40 cycles of denaturation at 95°C for 33 s, annealing at each gene-specific primer Tm (°C), and extension at 72°C for 33 s. Proper amplification of the genes of interest was verified by melting point analysis and 1.2% agarose gel electrophoresis.

Statistical analyses

All values represent as mean±SEM. The means were compared by Student's t-test and Tukey's Studentized Range (HSD) to detect significant differences. All the statistical analyses were performed with the SAS software package, ver. 9.0 (SAS, Cary, NC, USA).

RESULTS

Continuous growth of MSCs *in vitro*

MSCs isolated using trypsin-EDTA digestion were cultured in DMEM supplemented with 10% FBS at 37°C in an atmosphere of 5% CO_2. The primary MSC culture was maintained for 87 days and the changes in the cell culture were observed microscopically. MSCs proliferated and started to differentiate on day 8. Cells became confluent and myotubes became longer upon further culture. We expected the cells to differentiate and die after the cells get differentiated. In contrast, the cells grew continuously (Figure 1), which was consistent with the speculation that the population consisted of some cells that underwent rounds of division instead of undergoing differentiation. The capacity of MSC self-maintenance has been reported (Bischoff, 1994) and it has been speculated that asymmetric division of MSCs, dedifferentiation of myogenic cells and contribution from stem cells may be the mechanisms of self-renewal (Asakura et al., 2002; Zammit et al., 2004).

Expression of myogenin protein in myotube-formed cells

Results from the immunostaining of myogenin supported the differentiation into myotubes of isolated primary MSC cultures from bovine hind limb muscle (Figure 2). Myogenin antibody stained the differentiating MSCs on days 10 and 18; staining intensity was enhanced in myotube-formed cells on day 18. Myogenin antibody stained the nuclear part of the cells. Both real time RT-PCR and immunocytochemistry for myogenin showed that the isolated cells were MSCs that were capable of differentiating into myotubes *in vitro*.

Effect of steroid hormones on MSC proliferation

The effect of naturally occurring hormones in serum on cell proliferation was ascertained by subculturing cells in DMEM supplemented with 2% or 10% of male, female and castrated bovine serum. Primary culture was performed in DMEM supplemented with FBS. MSC proliferation was significantly enhanced in cultures supplemented with both 2% and 10% male serum compared to female and castrated serum ($p<0.001$ for 10% serum and $p<0.01$ for 2% serum; Tukey's test). Moreover, MSC proliferation was enhanced

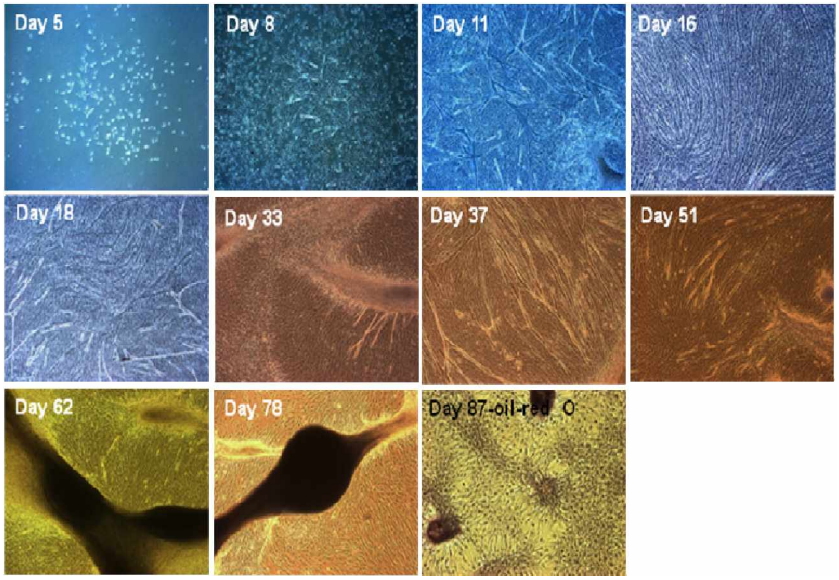

Figure 1. Proliferation and differentiation of MSCs. MSCs were isolated by digesting minced hind limb muscle in trypsin-EDTA and cultured in DMEM supplemented with FBS. Days on the left alignment of each picture represent the number of culture days of MSCs. Cells on day 5 were not differentiated. On day 8, cells had initiated myotube formation and myotubes were clearly visible on subsequent days. From day 33 onward, cell cluster-like structures were observed. The picture on day 87 was taken after Oil-Red-O staining.

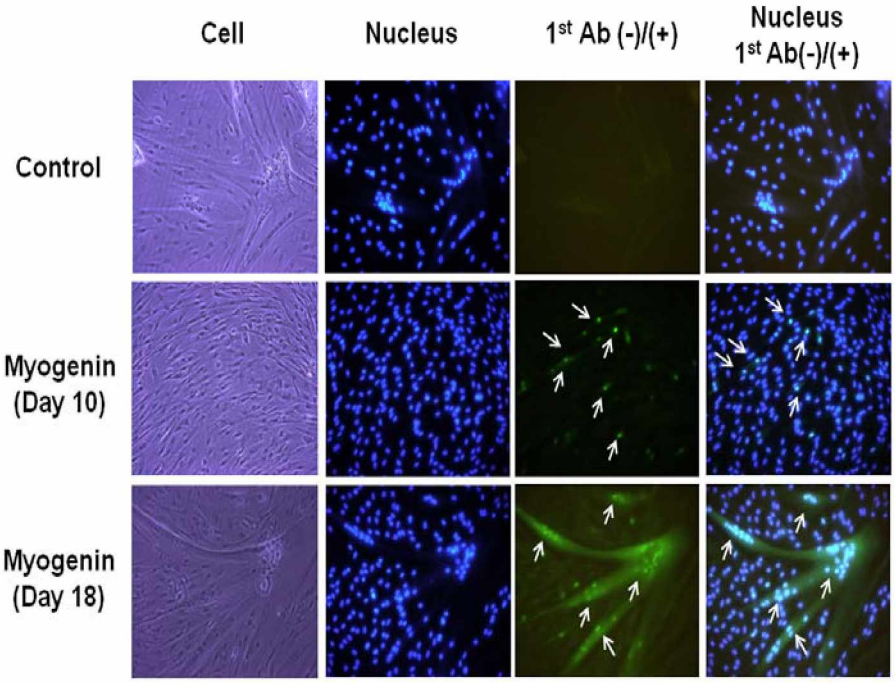

Figure 2. Immunocytochemistry. Differentiated MSCs were stained by mouse monoclonal IgG_1 myogenin followed by 4',6-diamidino-2-phenylindole (DAPI) staining for nucleus. The immunofluoroscence labeling was observed for the marker proteins of myotube (myogenin), particularly in the nuclear region of myotube-formed cells. Pictures: left lane shows cells, second lane shows DAPI-stained nuclei, third lane shows antibody fluorescence and the last lane shows merged DAPI-stained nuclei and antibody fluorescence pictures. Arrows on the pictures indicate nuclei stained with antibody. Control pictures are of cells without primary antibody treatment. Moreover, myogenin staining is higher in day 18 compared to day 10.

in medium supplemented with 10% serum compared to 2% serum ($p<0.05$; t-test). Cell proliferation in the culture medium supplemented with female and castrated serum was sequentially less avid than medium supplemented with male serum (Figure 3). The cell proliferation effect of male serum was further supported by the induced proliferation in the testosterone-treated culture supplemented with castrated serum. MSC proliferation was significantly higher in testosterone-treated medium supplemented with castrated serum ($p<0.01$; Tukey's test). Similar to the different serum conditions, cell proliferation was significantly higher in the 10% serum supplemented condition compared to the 2% serum supplemented condition ($p<0.05$). While, less cell proliferation was evident in E_2+testosterone- and E_2-treated cultures.

E_2 influence on lipid droplet accumulation in myotube

Successful transdifferentiation of MSCs using different adipogenic chemicals has been reported (Hu et al., 1995; Kook et al., 2006; Singh et al., 2007). Presently, to check whether steroid hormones influenced the accumulation of lipid droplets without treatment of transdifferentiation solution, isolated MSCs were cultured in the steroid-free condition prior to treatment of steroid hormones (testosterone, E_2 and E_2+testosterone). E_2-treated cell cultures showed the highest lipid droplet accumulation, followed by E_2+testosterone, testosterone alone and control (Figure 4).

Expression of myogenic marker genes and steroid receptors in MSCs and myotube-formed cells

MSCs were cultured for 9, 14 and 21 days. Total RNA was extracted separately and expression levels of different genes were ascertained. Higher expression of myogenin and desmin at 14 days rather than 9 days was an indication of myogenesis. Moreover, expression of myogenin and desmin was the highest in cells cultured for 21 days. From the same cDNA samples, real time RT-PCR was performed for observation of ER-α, AR and PR mRNA expression levels. Fold differences of the mRNA expression of all three species were significantly higher in 21 days cultured myogenic cells. Furthermore, there was no difference in ER-α expression, whereas AR and PR were induced during the formation of myotubes at 14 days compared to MSCs at 9 days of culture (Figure 5). The result indicates the possible role of steroids in differentiation of MSCs.

DISCUSSION

MSCs differentiate and fuse to augment existing muscle fibres and to form new fibres. After differentiation, MSCs

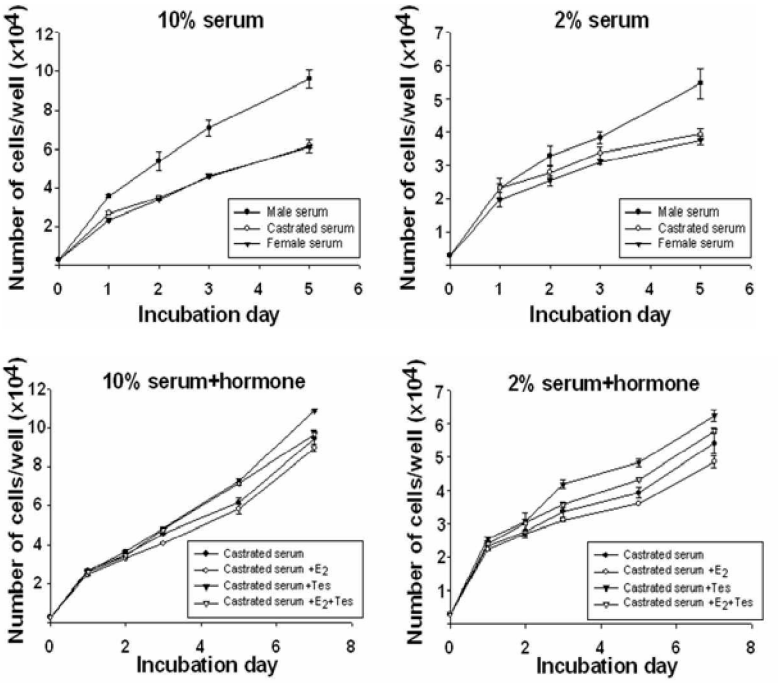

Figure 3. Effect of different serum and hormone treatments on cell proliferation of MSCs. MSC proliferation was observed in media supplemented with different adult serum (2% or 10%). Cells proliferation increased in male serum-supplemented media compared to the other sera. Furthermore, subcultured cells that were grown in DMEM supplemented with castrated bovine serum were treated with testosterone, E_2 or E_2 +testosterone. Cells proliferated highly in the testosterone-treated cultures. Data represent the mean±SEM.

form into myotubes and the differentiated cells do not divide. However, during *in vitro* culture of MSCs in DMEM supplemented with FBS, cells were presently observed to continuously grow; we maintained the cell culture until 87 days and growth could have continued longer. This result indicates that there might be a group of cells which proliferates by continuous cell division, instead of differentiation. Several papers have reported on the presence of quiescent MSCs that divide slowly or not at all unless certain external stimuli such as injury, myotube death or exercise are applied (Zammit and Beuchamp, 2001; Zammit et al., 2004). The hypothesis that self-renewal is a

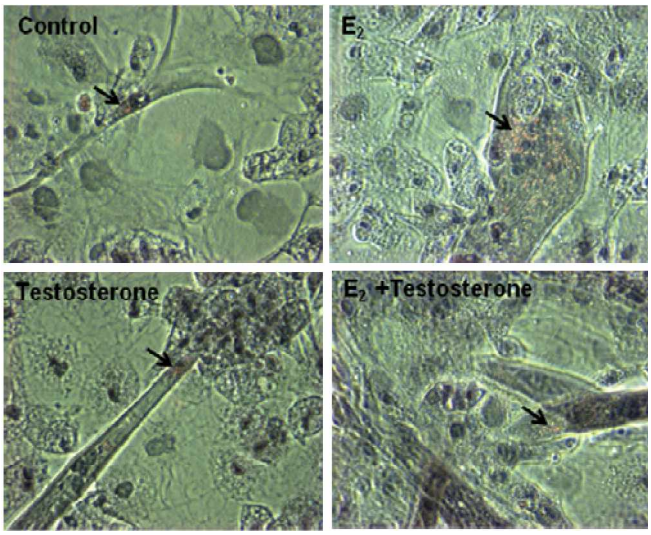

Figure 4. Effect of steroids on lipid accumulation in myotubes. Cells grown in DMEM supplemented with FBS were further cultured in steroid-free culture medium (DMEM without phenol red supplemented with CDFBS prior to steroid hormones (E_2, testosterone or E_2 and testosterone) treatment). Oil-Red-O/tri-ethyl phosphate stained the accumulated lipid droplets in myotube-formed cells. The highest lipid accumulation was observed in E_2-treated cultures. Arrows on the pictures depict Oil-Red-O stained lipid droplets. The pictures shown are the typical pictures from three experimental replicates.

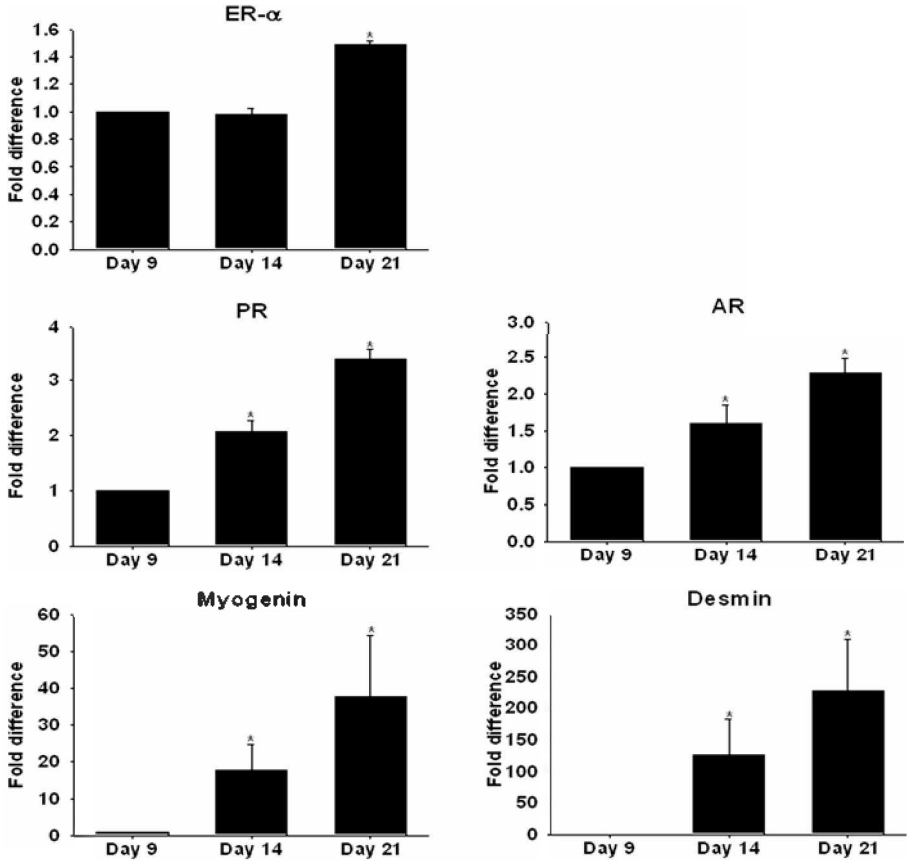

Figure 5. mRNA expression of different genes during MSC differentiation. Myogenesis, desmin and myogenin marker genes were up-regulated on days 14 and 21, compared with day 9. Similarly, expression of ERα, AR and PR ascended, while MSCs differentiated into myotubes on days 14 and 21. Expression of all the genes in 21 days was statistically different compared to those in myogenic cells at 9 days. Values represent mean±SEM (* $p<0.001$).

major mechanism that maintains the MSC cell pool (Moss and Leblong, 1971) has found experimental support (Zammit et al., 2004). However, presently the existence of a group of cells in MSCs that proliferate faster without undergoing differentiation remained to be clear.

The activation of MSCs from a state of quiescence and their progression along the myogenic differentiation are controlled by various transcription factors. MyoD appears during activation (Tajbakhsh et al., 1996). Desmin is the intermediate filament protein of muscle, which is expressed from the early formation of skeletal muscle (Costa et al., 2004). Myogenin is essential for muscle cell differentiation (Hasty et al., 1993) and is the marker protein of myogenesis localized in nucleus (Ishido et al., 2004). Therefore, immunocytochemical analysis was performed for the myotube marker protein myogenin to confirm that the isolated cells were MSCs. Microscopic observation of immunofluorescence confirmed the identity of the isolated cells as MSCs that formed myotubes after differentiation.

The effect of steroids on myogenesis of MSCs has been investigated. Our previous study also showed the importance of steroids in MSC growth (Lee et al., 2007). Testosterone induces muscle fiber hypertrophy with an increase in MSC number (Lee, 2002; Sinha-Hikim et al., 2003). Similarly, estrogen-stimulated augmentation of MSCs following exercise through ER-mediated mechanisms has also been reported (Enns et al., 2008). Here, the effect of natural hormones in the adult bovine serum (male, female and castrated serum) in MSC proliferation was observed. MSC proliferation was the highest in media supplemented with male serum followed by female and castrated serum, respectively. This may be because of the hormonal components present in the different sera. Hormonal analysis of the different sera detected high amounts of testosterone in male serum, estrogen and progesterone in female serum and no detectable level of these hormones in castrated serum (data not shown).

Steroid-free FBS has been used after treatment with charcoal-dextran to observe the effect of steroids in cell culture. However, charcoal-dextran removes growth factors along with steroids from the serum and cells do not grow well in CDFBS-supplemented media (McKeehan et al.,

1984). The present study adopted the novel track of using serum from castrated adult bovine as an alternative to CDFBS to observe steroidal effects during *in vitro* cell culture. MSC proliferation in the culture supplemented by castrated serum was enhanced by the sequential supplementation of testosterone and E_2. In one study, feedlot steers implanted with a combination of 120 mg synthetic androgen trenbolone acetate and 20 ng E_2 exhibited an increased rate of gain (20%-25%), increased feed efficiency (15%-20%), increased carcass protein and increased longissimus muscle area (Johnson et al., 1996).

It has been reported that the phytoestrogen, genistein, at high concentrations may stimulate adipogenic differentiation and cell number by acting as peroxisome proliferator-activated receptor-alpha agonist *in vitro* (Ricketts et al., 2005). In this study, we found the increase in lipid droplets accumulation in myotubes when treated with E_2 followed by E_2+testosterone and testosterone alone. In contrast to this result, male and female ER-α knockout mice display an increase in adipose tissue compared to wild-type mice indicating that E_2 normally has anti-lipogenic roles (Heine et al., 2000). Another study also showed that differentiated adipocytes accumulate smaller oil droplets suggesting a reduced extent of maturation in the presence of dihydrotestosterone *in vitro* (Gupta et al., 2008).

The effects of sex steroid hormones on muscle tissue including testosterone, androgen and estrogen on MSCs have been well studied (Inoue et al., 1994; Arnold et al., 1996; Kahlert et al., 1997; Lee, 2002; Sinha-hikim et al., 2003; Enns et al., 2008). In this study, to understand the molecular mechanism of the effect of hormones on the differentiation of MSCs, we analyzed the change in mRNA expression level of steroid receptors in MSCs and differentiated cells. Expression of AR and PR increased with myogenesis in 14 days while there was no change in ER-α expression. However, ER-α expression increased in RNA samples collected after 21 days, implying a late response of ER-α during myogenesis. These findings led us to speculate that the effects of testosterone and E_2 in MSC differentiation are mediated by their receptors. E_2-stimulation also occurs via different mechanisms like GPR30 receptors (Kamanga-Sollo et al., 2008a). Moreover, mRNA expression of desmin and myogenin was presently analyzed; the results support the protein expression data shown by immunocytochemical analysis.

Taken together, the present data demonstrates that steroid hormones are one of the key affecting factors in proliferation of MSCs and lipid accumulation in myotubes. In addition, this study highlights the possibility of the use of castrated adult serum as an alternative to CDFBS in studying the effect of steroids. Further studies are needed to elucidate the molecular mechanisms of the effect of steroid hormone on MSCs during proliferation and differentiation.

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