Satellite Cell Proliferation and Differentiation
During Postnatal Growth of Porcine Skeletal Muscle

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ABSTRACT

Age-related changes in satellite cell proliferation and differentiation during rapid growth of porcine skeletal muscle were examined. Satellite cells were isolated from hindlimb muscles of pigs at 1, 7, 14, and 21 weeks of age (4 animals/age group). Satellite cells were separated from cellular debris using Percoll gradient centrifugation, and adsorbed to glass coverslips for fluorescent immunostaining. Positive staining for neural cell adhesion molecule (NCAM) distinguished satellite cells from non-myogenic cells. The proportion of NCAM-positive cells (satellite cells) in isolates decreased from 1 to 7 weeks of age. Greater than 77% of NCAM-positive cells were proliferating cell nuclear antigen (PCNA)-positive at all ages studied. Myogenin-positive satellite cells decreased from 30% at 1 week to 14% at 7 weeks of age and remained at constant levels thereafter. These data indicate that a high percentage of satellite cells remain proliferative during rapid postnatal muscle growth. The reduced proportion of myogenin-positive cells during growth may reflect a decrease in the proportion of differentiating satellite cells or accelerated incorporation of myogenin-positive cells into myofibers.

Keywords: Proliferating Cell Nuclear Antigen, Myogenin, DNA accretion
**INTRODUCTION**

Postnatal skeletal muscle growth in the pig occurs through hypertrophy of existing muscle fibers, since the number of muscle fibers is determined prenatally and remains constant throughout postnatal life (37, 42, 43). Muscle fiber hypertrophy is associated with an increased DNA content, yet myonuclei do not retain the ability to synthesize DNA (38). Satellite cells, first discovered by Alexander Mauro in 1961 (25), are the postnatal source of DNA contributed to growing muscle fibers. DNA accretion occurs through proliferation of satellite cells followed by differentiation and fusion with existing muscle fibers (28).

It is generally accepted that the proportion of myonuclei that are satellite cells declines with age. Campion et al. (7) observed a decrease in the proportion of nuclei classified as satellite cells in histological sections of *peroneus longus* and *sartorius* muscles from pigs between 1 and 64 weeks of age. However, these authors also suggested that the absolute number of satellite cells in these muscles may increase from 1 to 32 weeks of age. Increases in the absolute number of satellite cells throughout postnatal growth have also been reported for *semimembranosus* muscles of Japanese quail (8) and *soleus* muscles of the rat (17). Mulvaney et al. (30) observed a decrease in the proportion of nuclei that incorporated $^3$H-thymidine in the *triceps brachii* muscle of pigs from 1 to 21 days old, and suggested that satellite cell proliferation declined during this period. Similarly, the proportion of muscle nuclei that are satellite cells and the percentage of muscle nuclei that exhibit DNA synthesis decreases with advancing age in mice and rats (1, 9, 36). Age-related changes in satellite cell proliferation throughout the rapid growth phase of porcine skeletal muscle have not been characterized.

Satellite cell differentiation is likely to modulate muscle growth, as it regulates accretion of DNA in muscle fibers as well as the number of satellite cells that remain capable of
proliferation. Quinn et al. (32) demonstrated that embryonic myoblasts isolated from fetal calves with the double-muscled phenotype exhibit a delay in differentiation compared to myoblasts isolated from normal fetuses. This delay in differentiation resulted in an enlarged population of myoblasts and an increased production of fused myotubes \textit{in vitro}. Coutinho et al. (13) observed a similar delay in formation of the brachial somites in quail embryos selected for rapid muscle growth. These data indicate that the timing of differentiation is a critical event in muscle development.

Age-related changes in satellite cell differentiation during postnatal skeletal muscle growth have not previously been examined. We characterized age-related changes in satellite cell proliferation and differentiation to provide insight into the dynamics of these activities during rapid skeletal muscle growth. These data will be useful in elucidating the cellular mechanisms governing skeletal muscle DNA accretion.

\textbf{MATERIALS AND METHODS}

\textit{Tissue Collection}

Duroc x Yorkshire x Landrace pigs at 1, 7, 14, and 21 weeks of age (4 animals per age group) were slaughtered at the Michigan State University Meat Laboratory following procedures outlined in the Code of Federal Regulations for Humane Slaughter of Livestock (sections 313.1-313.9). The right and left \textit{semitendinosus} (ST) muscles were excised, denuded and weighed. Satellite cells were isolated from the right ST muscles of 7, 14, and 21-week-old pigs as described by Doumit and Merkel (15). Briefly, muscles were excised, trimmed of visible connective tissue, sectioned and ground in an aseptically prepared meat grinder. Ground muscle was incubated for 50 min at 37°C in a solution of 0.8 mg/ml Protease (EC 3.4.24.31; Sigma lot #88H1351; 3.9 units/mg solid) dissolved in phosphate-buffered saline (PBS) (2 parts muscle:3
parts Protease solution v/v). Following enzymatic digestion, cells were separated from tissue fragments by repeated centrifugation at 300 x g for 5 min, followed by filtration through 500- and 53-µm mesh nylon cloth. Due to the quantity of muscle mass required for this procedure, the right ST and *semimembranosus* muscles, which exhibit similar growth patterns (14), were used for satellite cell isolation from 1-week-old pigs. Primary muscle cell isolates were stored in liquid nitrogen until use.

Samples from the left ST were cut into ~0.5 cm³ sections, frozen in liquid nitrogen and stored at -80°C. For determination of DNA and protein concentrations, 1.0 g of muscle was homogenized in 25 volumes (w/w) of cold extraction buffer (10 mM Tris, 5 mM EDTA, pH 8.0). Triplicate 100 µl and 250 µl aliquots of muscle homogenate were used for DNA and protein assays, respectively. Muscle DNA content was determined using the procedure of Labarca and Paigen (21). Calf thymus DNA (Sigma Chemical Co., St. Louis, MO) served as a standard. Muscle protein content was determined using the biuret procedure outlined by Gornall et al. (18) and modified by Robson et al. (33). Bovine serum albumin (Sigma Chemical Co., St. Louis, MO) served as the protein standard.

**Satellite Cell Enrichment and Adsorption to Coverslips**

Porcine satellite cells were separated from tissue debris using Percoll gradient centrifugation as described by Yablonka-Reuveni (45). Briefly, primary muscle cell suspensions derived from 3.5 g of fresh muscle were loaded onto gradients and centrifuged at 15,000 x g for 5 min. The interface between the 20% and 60% Percoll solutions was recovered, diluted and centrifuged at 300 x g for 15 min to pellet cells. Cells were re-suspended in 200 µl of Minimum Essential Medium and allowed to adsorb to 12 mm-diameter glass coverslips (Fisher Scientific,
Itasca, IL) for 45 min. For each satellite cell isolate, cells were adsorbed onto separate coverslips for PCNA and myogenin staining, respectively.

**Immunostaining for Neural Cell Adhesion Molecule (NCAM)**

Cells adsorbed to coverslips were incubated in PBS containing 2% goat serum (blocking solution) to block non-specific antibody binding. Cells were then incubated in undiluted 5.1H11 hybridoma supernatant or 1 µg/ml non-specific mouse IgG (negative control; Sigma Chemical Co., St Louis, MO) for 30 min. The 5.1H11 hybridoma, developed by F.S. Walsh, was obtained from the Developmental Studies Hybridoma Bank developed under the auspices of the National Institute of Child Health and Human Development (NICHD) and maintained by the University of Iowa, Department of Biological Sciences, Iowa City, IA. Monoclonal antibody 5.1H11 has been shown to specifically recognize a surface antigen (NCAM) on human (41) and porcine (5) myogenic cells. Following three washes, cells were incubated for 30 min with 0.4 µg/ml biotinylated goat anti-mouse IgG1 (Caltag Laboratories, Burlingame, CA) and the washing series was repeated. To detect primary antibody binding, cells were incubated for 30 min with 1:200 ExtrAvidin conjugated to tetramethylrhodamine isothiocyanate (TRITC) (Sigma Chemical Co., St Louis, MO). Cells were washed then fixed with 1% formalin in PBS for 10 min, followed by a 10-min exposure to -20°C methanol. The wash series was repeated and cells were stained for either the presence of PCNA or myogenin. All washes and antibody incubations were in blocking solution, unless otherwise specified. Representative immunostaining for NCAM is depicted in Fig. 1 (B, E).

**Immunostaining for Proliferating Cell Nuclear Antigen (PCNA)**

Porcine satellite cells were incubated in 1 µg/ml anti-PCNA monoclonal antibody (PC-10, Boehringer Mannheim, Indianapolis, IN) or 1 µg/ml non-specific mouse IgG (negative
control) for 1-2 h. Proliferating cell nuclear antigen is expressed in the G₁, S, and G₂ phases of the cell cycle (27, 6) and has been previously used as an index of satellite cell proliferation *in vitro* (20, 47). Cells were washed and incubated for 30 min in 0.8 µg/ml goat, anti-mouse IgG₂a conjugated to fluorescein isothiocyanate (FITC) (Caltag Laboratories, Burlingame, CA). The washing series was repeated and coverslips were mounted onto microscope slides with 2.5 µl of VectaShield (Vector Laboratories, Burlingame, CA) mounting medium containing 4’,6-diamidino-2-phenylindole (DAPI) to counterstain DNA. Coverslips were sealed with nail polish and cells were viewed using a Leica DMLB fluorescent microscope. Representative immunostaining for PCNA is depicted in Fig. 1 (C). At least 600 cells from each animal were evaluated at 400x magnification in random or sequential fields of view. For each field of view, total cell number (DAPI+), total proliferating (PCNA+) cells, total satellite cells (NCAM+), and proliferating satellite cells (NCAM+/PCNA+) were enumerated by two independent evaluators blinded to the identity of the sample. The numbers of non-myogenic (NCAM-) and proliferating non-myogenic (NCAM-/PCNA+) cells were calculated by difference.

*Immunostaining for Myogenin*

Porcine satellite cells were incubated in undiluted F5D hybridoma supernatant or 1 µg/ml non-specific mouse IgG for 1-2 h. The F5D hybridoma, developed by W.E. Wright, was obtained from the Developmental Studies Hybridoma Bank developed under the auspices of the NICHD and maintained by the University of Iowa, Department of Biological Sciences, Iowa City, IA. The F5D hybridoma cell line produces an antibody against myogenin, a skeletal muscle-specific transcription factor expressed immediately prior to and throughout differentiation (44, 3). Following primary antibody incubation, cells were washed 3 times in blocking solution then incubated for 30 min with goat anti-mouse IgG conjugated to FITC
(Sigma Chemical Co., St Louis, MO) diluted 1:100 in blocking solution. Cells were washed, mounted onto microscope slides and viewed as described above. Representative immunostaining for myogenin is depicted in Fig. 1 (F). At least 700 cells were evaluated at 400x magnification in random or sequential fields of view. Two independent evaluators, who were blinded to sample identity, enumerated total cells (DAPI+), satellite cells (NCAM+) and myogenin+ cells.

**Statistical Analysis**

Data were analyzed using Statistical Analysis Software (SAS Institute Inc., Cary, NC). Percentage data were converted to a decimal value and transformed as the inverse sine of the square root. A one-way Analysis of Variance (ANOVA) and Bonferroni multiple comparisons test was used to compare treatment means with a Type I error rate of 5%.

**RESULTS**

*Whole Body Weight and Muscle Characteristics.* Live weights for pigs at 1, 7, 14 and 21 weeks of age are shown in Fig. 2. *Semitendinosus* (ST) muscle weight increased in proportion to body weight with the most rapid increase in cumulative weight gain occurring between 14 and 21 weeks of age (Fig. 2). Total DNA and protein content of ST muscles (Fig. 3) parallel increases in ST muscle weight (Fig. 2).

*Percentage of Satellite Cells (NCAM+ Cells).* The percentage of NCAM+ cells declined ($P < 0.05$) in muscle isolates from pigs from 1 to 7 weeks of age then remained constant thereafter (Fig. 4). This indicates that the proportion of satellite cells to non-myogenic cells is highest in isolates from young pigs. Similar percentages of NCAM+ cells were observed on coverslips stained for the quantification of PCNA+ or myogenin+ cells (data not shown).

*Percentage of Proliferating Satellite Cells (PCNA+/NCAM+ Cells) and Non-Myogenic Cells (PCNA+/NCAM- Cells).* The percentage of proliferating satellite cells (PCNA+/NCAM+)
isolated from muscle of 14-week-old pigs was lower \( (P < 0.05) \) than that from muscle of 1-week-old pigs (Fig. 5). However, a high proportion (> 77%) of satellite cells were PCNA+ for all ages examined. The percentage of proliferating non-myogenic (NCAM-/PCNA+) cells was lower \( (P < 0.05) \) in 1-week-old pigs than in pigs 7 to 21 weeks of age (Fig. 6). No differences in the percentage of proliferating non-myogenic cells were detected in isolates from pigs at 7, 14, or 21 weeks of age.

**Percentage of Differentiating Satellite Cells (Myogenin+ cells).** All myogenin+ cells were also NCAM+, as would be expected if both markers are muscle cell specific. The percentage of myogenin+ satellite cells was highest \( (30.7 \pm 3.6\%\; P < 0.05) \) in 1-week-old pigs, and decreased to \( 14.4 \pm 1.2\% \) in 7-week-old pigs (Fig. 7). A slight numerical decline in the percentage of myogenin+ satellite cells was observed in muscle of pigs between 7 and 21 weeks of age (Fig. 7).

**DISCUSSION**

Age-related changes in populations of proliferating and differentiating satellite cells were characterized in growing pigs from 1 to 21 weeks of age. Satellite cells facilitate skeletal muscle DNA accretion through proliferation, followed by differentiation and fusion with existing muscle fibers. The positive relationship between number of myonuclei and myofiber size is well documented \( (28, 10, 11) \). Likewise, Powell and Aberle \( (31) \) demonstrated that in lines of swine differing in muscularity, increased muscle mass is associated with an increased DNA content. Quantification of proliferating and differentiating satellite cell populations during growth of large mammals has not been previously reported. Our results indicate that a reduced proportion of differentiating satellite cells and to a lesser extent, a reduced population of proliferating cells,
may modulate changes in size of the satellite cell population and myonuclear accretion during muscle growth.

Pigs were in a rapid state of whole body and muscle growth from 1 to 21 weeks of age, as indicated by increases in both live weight and ST muscle weight (Fig. 2). The ST muscle was chosen for use in this study because it can be removed quickly and quantitatively, and ST growth parallels changes in total body weight (Dr. R.A. Merkel, personal communication). Accumulation of muscle DNA paralleled increases in ST muscle weight (Fig. 2 and 3). Interestingly, the capacity of skeletal muscle to accumulate DNA appears to increase during growth. The proportions of ST muscle DNA present at 21 weeks of age that accumulate prior to 7 weeks, between 7 and 14 weeks, and from 14 to 21 weeks of age are 20.6%, 35.9%, and 43.5%, respectively.

In the current study, satellite cells were distinguished from non-myogenic cells based on reactivity with monoclonal antibody 5.1H11. This antibody specifically recognizes a cell-surface antigen (NCAM) on human (41) and porcine (5) myogenic cells, but not on non-myogenic cells derived from muscle. The proportion of NCAM+ cells in muscle isolates declines in muscle of growing pigs from 1 to 7 weeks of age (Fig. 4). A number of explanations can account for the observed increase in proportion of non-myogenic cells to satellite cells. A relatively high proportion of myogenin+ cells at 1 week of age (Fig. 7) indicates that a greater proportion of satellite cells are committed to differentiation and would subsequently fuse with myofibers and be removed from the extractable mononuclear cell population. Therefore, as a percentage of total cells isolated from muscle, the proportion of satellite cells would decrease relative to non-myogenic cells at the subsequent ages. It is also possible that changes in the levels of one or more mitogens in muscle may directly influence the proportion of satellite cells
to non-myogenic cells. Vandenburgh et al. (40) demonstrated that a 16- to 22-hour exposure of embryonic chicken muscle cultures to muscle extracts from 86-day-old mice increased the proportion of fibroblastic cells when compared to extracts from younger (28-day-old) mice. It is interesting to note that the percentage of proliferating non-myogenic cells (PCNA+/NCAM-) also increased at the later time points examined in this study (Fig. 6). Mitogens released from crushed extracts of rat muscle have been shown to stimulate the proliferation of satellite cells but not muscle derived fibroblasts (4, 12). Expression of an active component of crushed muscle extract, hepatocyte growth factor (HGF) (39), decreases during development of rat skeletal muscle (19). It is possible that levels of HGF decrease throughout growth of porcine skeletal muscle or satellite cells become unresponsive to HGF, thereby decreasing the proportion of myogenic to non-myogenic cells.

It is generally accepted that at birth a high proportion of satellite cells exist in a proliferative state and as an animal ages this proportion decreases as satellite cells enter a quiescent state (2). Age-related declines in the proportion of muscle nuclei classified as satellite cells have been reported for the pig (7), mouse (9) and rat (1). Decreased $^3$H-thymidine and 5-bromo-2'-deoxyuridine (BrdU) labeling of nuclei in muscle during growth has been reported for young pigs (30) and growing turkeys (29), respectively. These studies report labeled nuclei as a percentage of total muscle nuclei, which includes both myofiber nuclei and satellite cell nuclei. Since the number of myonuclei per muscle fiber increases throughout postnatal growth (28), it is difficult to determine if a decline in DNA labeling index represents an absolute decrease in the proportion of proliferating satellite cells.

Proliferating cell nuclear antigen was used to detect proliferating satellite cells in the current study. This antigen is undetectable in quiescent cells via immunofluorescence when
methanol is used as a fixative, but is up-regulated in the G\textsubscript{1} phase of the cell cycle, peaks during S phase and declines during G\textsubscript{2} (27, 6). A number of other studies have employed immunostaining against PCNA to quantify the index of proliferation for satellite cells \textit{in vitro} (20, 46, 47). Nuclei of proliferating, clonally-derived porcine satellite cells (16), but not nuclei of porcine satellite cell-derived myotubes, are PCNA immunoreactive (unpublished observations). Expression of PCNA is also correlated to DNA synthesis and is well suited for use as an index of proliferation \textit{in vivo}, particularly when used in combination with muscle-specific markers (22). In the current study, all cells that exhibited PCNA immunostaining above negative control staining were scored as PCNA+. Although no attempt was made to quantify variation in fluorescent staining intensity, proliferating satellite cells from 14- and 21-week old pigs exhibited more heterogeneous PCNA immunostaining (a greater number of weakly immunoreactive nuclei) compared to PCNA+ satellite cells from younger animals (unpublished observations). Since PCNA expression depends on the phase of the cell cycle, it is tempting to speculate that the heterogeneous staining observed in older pigs may be due to an increased proportion of slowly cycling satellite cells. Although PCNA immunostaining does not allow determination of cell cycle time, it is compatible with detection of other antigens and relatively inexpensive compared to disposal costs incurred following administration of \textsuperscript{3}H-Thymidine or BrdU to large animals.

We observed a relatively modest decline in the proportion of PCNA+ satellite cells during muscle growth of pigs (Fig. 5). Furthermore, more than 77% of satellite cells isolated from 21-week-old pigs are PCNA+. These cells were presumed to be in a proliferative state at the time of isolation, although it is conceivable that some PCNA could be synthesized during cell isolation and NCAM staining prior to fixation (~8 h in a thawed, unfixed state). The high
proportion of PCNA+ satellite cells observed is not unexpected, since ST muscle weight, DNA and protein increase steadily from 1 to 21 weeks of age. Our findings are in agreement with those of Schultz (35), who reported that 80% of satellite cells from 30-day-old growing rats proliferate with a 32-hour cell cycle as determined by dual-labeling experiments utilizing $^{3}$H-Thymidine and BrdU. The remaining 20% proliferate at a much slower rate, possibly entering a quiescent state between divisions (35). The PCNA labeling method utilized in the current study is more likely to positively identify rapidly growing cells than slowly growing cells. The proportion of PCNA+ satellite cells observed from muscle of growing pigs at different ages is similar to the proportion of rapidly cycling satellite cells of growing rat muscle.

Little information exists on the age-related changes in satellite cell differentiation during muscle growth *in vivo*. Myogenin was used to establish an index of differentiation in the current study because it is up-regulated relatively early in the myogenic differentiation program (3). Myogenin expression also precedes irreversible cell cycle withdrawal (3). Cultured porcine satellite cells that have been induced to differentiate, as well as porcine satellite cell-derived myotubes, exhibit positive nuclear myogenin immunofluorescence (unpublished observations). In the current study, myogenin and PCNA are co-expressed, since the percentage of PCNA+ and myogenin+ cells total more than 100% for 1-week-old pigs. Approximately $31 \pm 3.6\%$ of satellite cells isolated from 1-week-old pigs are myogenin+ and this percentage declines to approximately $9 \pm 0.3\%$ at 21 weeks of age (Fig. 7). Clearly, the proportions of isolated myogenin positive cells reported herein can only be used as indices of satellite cell differentiation, rather than absolute values, since isolated satellite cells do not include differentiated satellite cells that are undergoing fusion with the sarcolemma. Nonetheless, our findings corroborate the conclusions of Schultz (34), who examined the satellite cell population
in mice from 7 to 30 d of age. Schultz (34) demonstrated a high percentage incorporation of satellite cells in very young mice and suggested that the incorporation of satellite cells decreases with age.

In the current study, the majority of muscle DNA accumulation occurred between 7 to 21 weeks of age, a time during which the proportion of myogenin+ satellite cells is relatively low (Fig. 7). A reduced proportion of differentiating satellite cells, combined with high index of proliferation, may increase the absolute number of satellite cells during muscle growth. Although the absolute number of satellite cells were not quantifiable using the methods employed in the current study, increases in the absolute number of satellite cells throughout postnatal growth have been suggested for the peroneus longus and sartorius muscles of the pig (7), the semimembranosus muscle of Japanese quail (8) and the soleus muscle in the rat (17). More recently, Yablonka-Reuveni et al. (47) demonstrated that cultured myofibers from old rats support more proliferating MyoD+ satellite cells than myofibers from young rats, suggesting that the number of myogenic precursors increases with age. Thus, it appears that a relatively large population of satellite cells may exist in the muscle of older animals and the reduced rate of differentiation may facilitate expansion of the satellite cell population. Despite the reduced percentage of myogenin+ satellite cells, the absolute number of satellite cells incorporating into muscle fibers may remain high due to an enlarged satellite cell population.

An alternative explanation for the reduced percentage of myogenin+ cells is that the rapidly growing satellite cells from younger animals may transition into myogenin positive states more quickly than those from older animals. Yablonka-Reuveni et al. (47) observed that the increase in number of proliferating satellite cells and transition into the myogenin positive state was more rapid for cultured satellite cells (in association with muscle fibers) from young rats.
compared to older rats. It is possible that satellite cells from younger animals become myogenin positive more readily, but do not incorporate into muscle fibers as rapidly as satellite cells from older animals. This could explain why a higher proportion of myogenin+ satellite cells exists in 1-week-old pigs compared with 7- to 21-week-old pigs, despite the relative increase in muscle DNA accumulation that occurs from 7 to 21 weeks of age. It is not clear whether the reduced proportion of myogenin+ cells in 7- to 21-week-old pigs reflects a decrease in the proportion of differentiating satellite cells, a slower transition of cells into the myogenin positive state, an accelerated rate of incorporation of myogenin+ cells into myofibers, or a combination of the latter.

Differentiation and incorporation of satellite cells into muscle fibers provides only one mechanism to control the satellite cell population. Another possible satellite cell fate is programmed cell death, or apoptosis. Apoptosis has been observed in satellite cell lines in vitro in response to serum starvation or staurosporine treatment (23, 24, 26). It is unknown if porcine satellite cells or incorporated myonuclei undergo significant amounts of apoptosis throughout postnatal growth. However, apoptosis may be a mechanism to control the size of a satellite cell population that contains a high proportion of proliferating and a decreasing proportion of differentiating cells as observed in this study.

Our present results suggest that a high proportion of proliferating satellite cells persist in muscle of rapidly growing pigs from 1 to 21 weeks of age, whereas an age-related decline in the proportion of myogenin+ satellite cells occurs relatively early in postnatal growth. Decreased satellite cell differentiation may result in an enlarged satellite cell population and increased capacity for myonuclear accretion.
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**Fig. 1.** Fluorescence micrographs of muscle-derived cells. Isolated cells from muscle of 1-week-old pigs were subjected to double immunofluorescence labeling for the presence of NCAM and PCNA or myogenin. *(A, D):* Total nuclei were visualized with DAPI. *(B, E):* Immunostaining for NCAM (satellite cell marker). *(C):* PCNA immunofluorescence labeling. *(F):* Myogenin immunofluorescence labeling. In each case, closed and open arrows indicate positive and negative staining, respectively. Bar = 50 µm.

**Fig. 2.** Changes in mean live weight (solid diamond) and mean semitendinosus (ST) muscle weight (open circle) of pigs at 1, 7, 14 and 21 weeks of age. Error bars represent standard error of the mean (SEM). When not visible, error bars are smaller than the marker.

**Fig. 3.** Changes in mean total DNA (solid diamond) and mean total protein (open circle) contents of the semitendinosus (ST) muscle of pigs at 1, 7, 14, and 21 weeks of age. Error bars represent standard error of the mean (SEM). When not visible, error bars are smaller than the marker.

**Fig. 4.** Percentage of NCAM+ cells (satellite cells) isolated from muscle of growing pigs at 1, 7, 14, and 21 weeks of age. Mean value ± SEM are plotted with different superscripts indicating means that differ (P <0.05).

**Fig. 5.** Percentage of NCAM+/PCNA+ cells (proliferating satellite cells) isolated from muscle of growing pigs at 1, 7, 14, and 21 weeks of age. Mean value ± SEM are plotted with different superscripts indicating means that differ (P <0.05).
**Fig. 6.** Percentage of NCAM-/PCNA+ cells (non-myogenic proliferating cells) isolated from muscle of growing pigs at 1, 7, 14, and 21 weeks of age. Mean value ± SEM are plotted with different superscripts indicating means that differ (P <0.05).

**Fig. 7.** Percentage of myogenin+ cells (differentiating satellite cells) isolated from muscle of growing pigs at 1, 7, 14, and 21 weeks of age. Mean value ± SEM are plotted with different superscripts indicating means that differ (P <0.05).
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