

Isolation and culture of wapiti (*Cervus elaphus*) satellite cells

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Burton, N. M., Shipley, L., Byrne, K. M., Vierck, J. L. and Dodson, M. V. 2000. **Isolation and culture of wapiti (*Cervus elaphus*) satellite cells.** *Can. J. Anim. Sci.* **80**: 303–309. Myogenic satellite cells (SC) were isolated from the sternomandibularis muscles of two, 227 kg, male wapiti (*Cervus Elaphus*) and studied in primary cell culture. Wapiti-derived SC were capable of attaching to culture substrata and following the myogenic program of proliferation and differentiation to form multinucleated myotubes. Wapiti SC attached equally well to pig skin gelatin (PSG), fibronectin (FN), Matrigel® and plastic ($P > 0.05$), but cell viability measured at 120 h varied depending on initial substratum type. Pig skin gelatin (0.02% wt vol⁻¹) was chosen for the majority of subsequent experimentation for cost efficiency. The greatest amount of wapiti SC proliferation was observed in media containing 10% (vol/vol⁻¹) horse serum (HS), 15% HS and 15% fetal bovine serum (FBS) ($P > 0.05$). Wapiti SC proliferated more when exposed to HS and FBS than to sheep serum (SS) ($P < 0.05$). No proliferation, differentiation or decrease in cell viability was observed in Dulbecco's Modified Eagle Medium (DMEM) + 1% HS, DMEM + 2% HS, DMEM + 3% HS or DMEM + 4% HS ($P > 0.05$) after 120 h in vitro. Proliferation of SC was doubled when insulin was added to both 10% HS- and 2% HS-containing media ($P < 0.05$). Although insulin alone in serum-containing media did not promote fusion of wapiti SC, two defined media (ITT and ITT-CF) that contain insulin did promote fusion of wapiti SC cultures. ITT-CF induced 3% fusion of wapiti SC into myotubes, and ITT induced 1% ($P < 0.05$). There was also an increase in total cell numbers in SC exposed to ITT-CF in comparison with ITT, ovine defined media (ODM) or ovine defined media-Modified (ODM-Mod) ($P < 0.05$). Although defined media differed in their ability to induce proliferation or differentiation ($P < 0.05$), the substrata on which the SC were plated did not influence the defined media effect on SC activity ($P > 0.05$). Satellite cells exposed to ITT and ITT-CF differed morphologically from SC exposed to ODM and ODM-Mod, which may suggest that formulation differences are influencing wapiti-derived SC proliferation and differentiation.

Key words: Wapiti, satellite cells, primary culture, myotubes

Burton, N. M., Shipley, L., Byrne, K. M., Vierck, J. L. et Dodson, M. V. 2000. **Isolement et mise en culture des cellules satellites de cerfs wapiti (*Cervus elaphus*).** *Can. J. Anim. Sci.* **80**: 303–309. Des cellules satellites (CS) myogènes isolées du muscle sternomandibularis de deux wapitis mâles (*Cervus elaphus*) de 227 kg ont été examinées en culture primaire. Les cellules étaient capables de se fixer sur les substrats de culture et, après une phase myogène de prolifération et de différenciation, de former des myotubes plurinucléés. Des cellules adhéraient tout aussi bien à la gélatine de peau de porc (GPP), à la fibronectine (FN), au Matrigel® et au plastique ($P > 0,05$), mais leur viabilité après 120 h variait selon le type initial de substrat. C'est la gélatine de peau de porc (0,02 % en poids/volume) qui, pour des raisons de rendement économique, a été retenue pour la majorité des essais subséquents. La prolifération la plus abondante des cellules était obtenue dans des milieux de culture contenant 10 % ou 15 % en volume de sérum de cheval (SCh), et 15 % de sérum de bovin fœtal (SBF), $P > 0,05$. La prolifération était plus abondante ($P < 0,05$) en présence de SCh et SBF qu'en présence de sérum de mouton (SM). Il n'y avait ni prolifération ni différenciation ni perte de viabilité des cellules ($P > 0,05$) au bout de 120 h en culture, dans le milieu de l'aigle modifié de Dulbecco contenant 1, 2, 3 ou 4 % de SCh. L'incorporation d'insuline à chacun des milieux contenant 10 et 2 % SH provoquait une augmentation du double de la prolifération cellulaire ($P < 0,05$). Bien qu'à elle seule l'insuline présente dans les milieux contenant du sérum n'ait pas stimulé la fusion de CS, deux milieux définis, ITT et ITT-CF, contenant de l'insuline suscitaient la fusion des CS en culture. ITT-CF suscitait en outre 3 % de fusion de CS en myotubes et ITT 1 % ($P < 0,05$). On observait également une augmentation des numérations totales de cellules en présence de ITT-CF par rapport à ITT, ODM (milieu ovin défini) ou ODM modifié ($P < 0,05$). Bien que les milieux définis différaient quant à leur aptitude à induire la prolifération ou la différenciation ($P < 0,05$), les substrats sur lesquels les CS étaient mis en culture n'avaient pas d'influence sur l'effet des milieux définis sur l'activité des CS ($P > 0,05$). Les CS mis en présence de ITT et de ITT-CF différaient au plan de la morphologie de celles exposées à ODM et à ODM modifié, ce qui laisse à conclure que les différences de formulation des milieux de culture influent sur la prolifération et sur la différenciation des CS.

Mots clés: Wapiti, cellules satellites, culture primaire, myotubes

Abbreviations: DMEM, Dulbecco's modified Eagle medium; DMSO, dimethyl sulfoxide; FBS, fetal bovine serum; FGF, fibroblast growth factor; FN, fibronectin; GDW, glass distilled water; HS, horse serum; IGF-I, insulin-like growth factor-I; ITT, insulin, T₃, transferrin; ITT-CF, insulin, T₃, transferrin, hydrocortisone, FGF; ODM, ovine defined media; ODM-Mod, ovine defined media-modified; PBS, phosphate buffered saline; PSG, pig-skin gelatin; SC, satellite cells; SS, sheep serum

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Interest in farming wild ruminants in North America is increasing, and today there are approximately 90 000 such animals valued at \$180 million on game farms in Canada (Hudson et al. 1989). Although deer are the most common animals on game farms, there are about 11 000 wapiti (*Cervus elaphus*) currently being farmed throughout Canada (Hudson et al. 1989). Wapiti display high growth rates, good carcass composition and dressage percentages between 55 and 58%. The high-priced cuts of the hindquarters and saddle of wapiti make up more of the carcass than in conventional meat animals (Haigh et al. 1993). Wapiti meat is also lean, palatable and commands a premium over beef (Haigh et al. 1993).

Traditional livestock producers have been aided by research aimed at understanding development characteristics of agricultural animals. Knowledge of postnatal skeletal muscle growth has increased with the discovery of satellite cells and their function in myogenesis. Satellite cell research has been conducted on a variety of agricultural animals (Dodson et al. 1986; Yablonka-Reuveni et al. 1987; McFarland et al. 1988; Powell et al. 1989; Greene et al. 1990; Doumit and Merkel 1992), and it has aided in increasing production and performance of these animals. Satellite cell research used in conjunction with wapiti and other ranched game animals will not only assist producers of these animals in maintaining viable herds, but may also give insight into possible similarities and differences between the growth and development of domesticated and "wild" animals.

Whole-animal growth dynamics have been a focus of wildlife research and management, but most studies have been limited to observing morphological changes in animals, correlating animal growth to environmental conditions (Webster et al. 1996a,b, Webster et al. 1999) or relating serum contents to periods of analysis of animal growth (Adam et al. 1996). The objective of this project, however, was to identify and characterize properties of wapiti-derived SC by isolating, culturing and defining the *in vitro* growth of wapiti SC.

MATERIALS AND METHODS

Animals

Two, 2-yr-old, male wapiti, each weighing 227 kg, were used for this study. Thirty days after birth, both animals were vaccinated with polyvalent and clostridial vaccines for respiratory and digestive disorders. Each was administered vitamins A and D and selenium during the fall of its first year and was provided with ivermectin (for internal parasites) regularly. Both had been fed an *ad libitum* diet of forage and alfalfa grass hay. Both animals were sexually mature but had not yet attained full adult, male stature. Animal handling and care were consistent with the guidelines laid down by the Canadian Council on Animal Care (1984).

Muscle Isolation

Dissection to remove both sternomandibular muscles was performed on the wapiti at the Washington State University Meat Science Laboratory. Each animal was restrained in a

commercial stunning chute that had been modified to minimize stress and was stunned with a .22 caliber rifle shot behind the right eye. Each was suspended vertically and then exsanguinated. The dorsal surface of the neck was shaved, and an incision was made along the length of the neck between the sternomandibular muscles. The muscles were exposed by carefully retracting the hide to the sides and back. With the hide retracted, the sternomandibular muscles were dissected from surrounding tissue by cutting them free from their insertions (the ramus of the mandible) and origins (the first costal cartilage). The excised muscles were immediately submerged in sterile phosphate buffered saline (PBS) (pH 7.08) supplemented with 10 000 units penicillin 10 mg⁻¹ streptomycin mL⁻¹. Approximately 10 min elapsed between the time of death and the placement of the sternomandibular muscles in PBS for transport to the cell culture laboratory.

Isolation of Satellite Cells

Wapiti-derived SC were isolated from the sternomandibular muscles as described in Burton et al. (1999). In brief, the muscles were rinsed with sterile PBS supplemented with antibiotics to remove any surface contamination. The muscles were then minced in a sterile meat grinder (1.5-mm plate) to increase the surface area of the tissue. Twenty grams (each) of minced muscle were placed into eight sterile, 50-mL centrifuge tubes. Pronase (Sigma Chemical Co. St Louis, MO; 1 mg mL⁻¹, dissolved in sterile PBS) was added to each tube up to 35 mL (Dodson et al. 1987). Each tube was vortexed and then incubated for 40 min at 37°C with agitation every 10 min. After 40 min, the cell/pronase mixture was centrifuged at 1500 × *g* for 10 min (Dodson et al. 1987). The supernatants were discarded, and sterile PBS was added up to 35 mL for each tube. The pellets were resuspended, and SC were isolated by differential centrifugation at 400 × *g* (Dodson et al. 1987). Isolated SC were aliquoted into 15-cm culture dishes coated with PSG (0.02% wt vol⁻¹) and exposed to DMEM + 10% FBS and antibiotics (10 000 units penicillin, 10 mg streptomycin, 10 mg amphotericin, and 10 mg gentamicin mL⁻¹). Plated cells were maintained in a humidified CO₂ incubator containing 95% (vol vol⁻¹) air and 5% (vol vol⁻¹) CO₂ at 37°C. When the stock plates reached 60% confluency, the cells were removed from the plates with trypsin, transferred into DMEM + 20% HS and 10% DMSO, and aliquoted into cryovials at 1 × 10⁶ cells/vial. The cryovials were left overnight in a biofreezer at -80°C and then transferred to liquid nitrogen (Molnar and Dodson 1993).

Cell Culture

Cells were plated at a density of either 20 SC mm⁻² or 100 SC mm⁻² per 16-mm-diameter cell culture well and allowed to attach for 24 h prior to the addition of test media. Treatments were applied to six replicate wells, except in the initial HS study in which 72 and 120 replicate wells were used. At the end of each experiment, cultures were fixed in absolute methanol and stained with Giemsa (Dodson et al. 1985). Proliferation and differentiation were evaluated by counting total and fused nuclei in 10 random observations per well at 400 × magnifications (Dodson et al. 1985).

Substratum Optimization

To identify a substratum that maximized attachment of wapiti SC, four attachment surfaces were screened. Substrata tested were: PSG [0.1 g, 0.5 g, 1 g 500 mL⁻¹ glass distilled water (GDW)], bovine FN (0.005 g, 0.01 g, 0.02 g 7 mL⁻¹ DMEM) and Matrigel® (1:5 DMEM ratio, 1:10 DMEM ratio, 1:15 DMEM ratio) (Dodson and Mathison 1988; Dodson et al. 1990; Venkateswaran et al. 1995). Uncoated, plastic wells were used in each experiment for comparison purposes. Cells were plated at 20 SC mm⁻² in either DMEM + 10% HS or 10% FBS for 24 h.

Media Characterization

DMEM + 10% HS promotes and maintains SC growth and viability in a number of animal cell culture systems (Dodson et al. 1986, 1987; McFarland et al. 1988; Greene et al. 1995). In order to compare wapiti-derived SC with other SC systems, wapiti SC were first examined for the variable of proliferation. Wapiti SC were plated in DMEM + 10% HS at 20 SC mm⁻² in 72 replicate wells and at 100 SC per mm⁻² in 120 replicate wells for a total of six experiments. Cultures were maintained for 120 h with replenishment of media every 24 h (Dodson and Mathison 1988).

Experiments were conducted to identify which of three sera added at different levels to basal media promoted proliferation of wapiti SC. Satellite cells were dispersed in DMEM plus HS, FBS or SS at four different concentrations (2, 5, 10 or 15 vol vol⁻¹). The SC were then plated onto PSG-coated, 24 well cell culture plates at a density of 20 SC mm⁻². Cultures were allowed to proliferate to 120 h with replenishment of media every 24 h. Satellite cell proliferation in the different media was determined by fixing treatment wells with methanol, staining with Giemsa and evaluating total nuclei density (Dodson and Mathison 1988).

Decreasing the amount of serum in basal media has been observed to initiate myoblast fusion in other systems (Dodson et al. 1987; McFarland et al. 1988; Doumit and Merkel 1992). To study wapiti-derived SC differentiation dynamics, SC were plated at 100 SC mm⁻² in DMEM + 10% HS into PSG-coated culture wells. After a 24-h attachment period, cultures were washed with sterile PBS. Treatment media of DMEM + 1% HS, DMEM + 2% HS, DMEM + 3% HS or DMEM + 4% HS were added. Cells were exposed to these fusion-induction media for 120 h.

Insulin has been observed to affect both the proliferation and differentiation activity of SC of many animal models (Doumit and Merkel 1991; Greene and Allen 1990; Ridpath et al. 1984). The effects of insulin on wapiti SC activity were examined by adding three insulin levels (0.1 μM, 0.5 μM, 1 μM) to DMEM + 10% HS or DMEM + 2% HS. After the initial 24-h attachment period, SC were exposed to treatments for 120 h, then proliferation and differentiation were evaluated (Dodson and Mathison 1998).

Four defined media were tested for their ability to induce differentiation in wapiti SC. The defined medium ITT was effective in promoting differentiation of sheep SC (Vierck et al. 1995), ITT-CF promoted differentiation of equine SC (Erickson et al. 1988), and ODM was developed

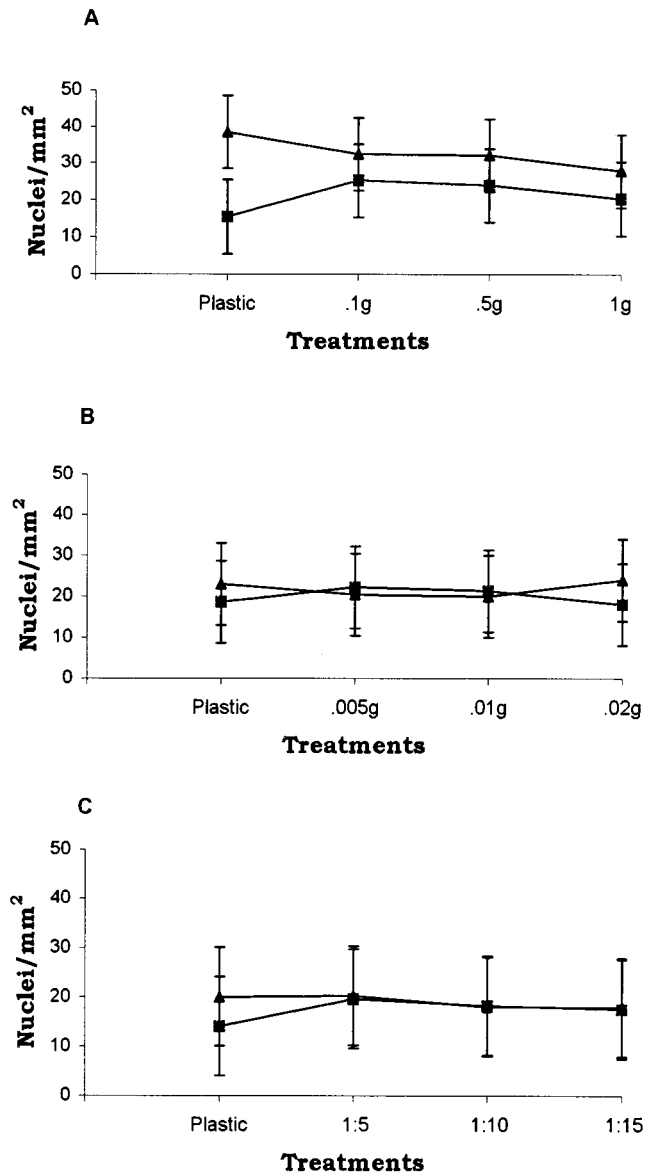


Fig. 1. Plating efficiency of wapiti SC. Cells were plated at 20 nuclei mm⁻² into 16 mm-diameter culture wells that were either uncoated or coated with a specific substratum and evaluated 24 h later for attachment characteristics. Concentrations of each substratum were: (A) PSG: 0.1 g, 0.5 g, 1 g 500 mL⁻¹ GDW; (B) FN: 0.005 g, 0.01 g, 0.02 g 7 mL⁻¹ DMEM; and (C) Matrigel®: 1:5 DMEM dilution, 1:10 DMEM dilution, 1:15 DMEM dilution. Each substratum concentration was exposed to either 10% HS (■-■) or 10% FBS (▲-▲). Each observation represents the sum of six treatment wells (\bar{x} = SEM); 10 randomly chosen microscopic fields were evaluated per well.

to maintain sheep-derived SC numbers (i.e. no proliferation or differentiation) in culture (Dodson and Mathison 1988). For this experiment, ODM was also modified (ODM-Mod) for the wapiti system to contain reduced levels of fibroblast growth factor (FGF) (from 20 μg to 5 μg) and increased levels of insulin (from 10⁻⁹ M to 10⁻⁶ M). Satellite cells were

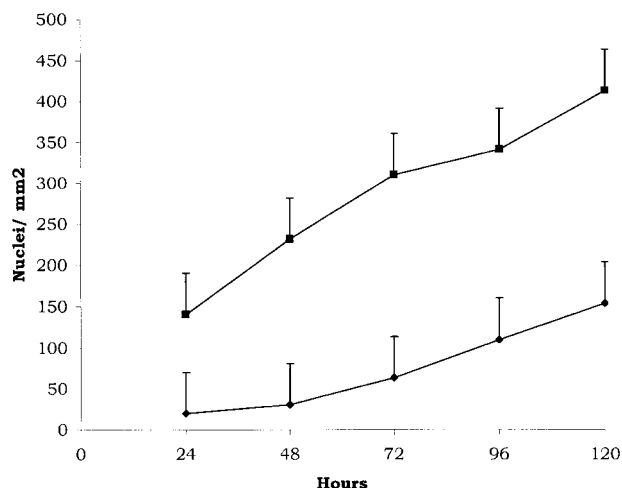


Fig. 2. 10% HS growth curves for wapiti SC. Cells were plated at either 20 nuclei mm⁻² (◆-◆) or 100 nuclei mm⁻² (■-■) in 75- and 120-, 16-mm-diameter culture wells, respectively. Cells were exposed to 10% HS for 120 h with media changes every 24 h. Cultures were then fixed, stained and evaluated.

plated at a density of 100 nuclei mm⁻² onto wells coated with either PSG (0.1 g 500 mL⁻¹ GDW) or FN (0.01 g 7 mL⁻¹ DMEM) in DMEM + 10% HS for 24 h. After this attachment period, the wells were washed, and defined media treatments were added. Satellite cells were exposed to treatments for 120 h, with treatments replenished every 24 h.

Statistical Analysis

All experiments were completely randomized designs. Each analysis of variance was conducted using MiniTab™ version 11.12. Development variables of the first experiment were four substrata and two media with one (two-way) interaction of all independent variables (substrata × media). Dependent variables of the second experiment were analyzed using a randomized model that included three serum types at four concentrations every 24 h for 120 h. Four (two- and three-way) interactions of all independent variables were tested (serum × concentration; serum × hours; concentration × hours; serum × concentration × hours). Dependent variables of the remaining experiments examined: 1) three insulin concentrations in either DMEM 2% HS or 10% HS every 24 h for 120 h and 2) four defined media on two substrata types at the end of 120 h. Four (two- and three-way) interactions of all independent variables of the insulin experiment (insulin × media; insulin × hours; media × hours, insulin × media × hours) and one interaction of all independent variables of the defined media experiment (defined media × substrata) were tested. Dependent variables of the effect of HS on fusion were analyzed using four low concentrations of HS every 24 h for 120 h. One (two-way) interaction of all independent variables was tested (concentration × hours). Initial analysis of means was conducted through one-way and two-way ANOVA. Significant treatment effects were calculated at $P < 0.05$ (Ott 1988).

Table 1. Mean (± SE) of the proliferation of wapiti satellite cells (nuclei mm⁻²) in three serum-containing media: HS, FBS and SS at four concentrations (DMEM medium + 2%, DMEM + 5%, DMEM + 10%, DMEM + 15%) after 120 h of culture

Concentrations	HS	FBS	SS	Pooled error
2%	11 ± 2.5 ^z	20 ± 6 ^y	7 ± 2	± 2
5%	25 ± 4 ^{y,x}	41 ± 11 ^w	14 ± 3 ^{xy}	± 5
10%	74 ± 16 ^v	47 ± 8 ^w	24 ± 3 ^{xu}	± 10
15%	96 ± 22 ^v	87 ± 20 ^v	28 ± 4 ^u	± 14

SC showed differences ($P < 0.05$) in their proliferative properties in all treatment combinations, except where indicated as follows:

^z2% HS and 5% SS ($P > 0.05$).

^y2% FBS and 5% HS or 5% SS ($P > 0.05$).

^x5% HS and 10% SS ($P > 0.05$).

^w5% FBS and 10% FBS ($P > 0.05$).

^v10% HS and 15% HS or 15% FBS ($P > 0.05$).

^u10% SS and 15% SS ($P > 0.05$).

RESULTS AND DISCUSSION

Techniques used to isolate SC from the sternomanibular muscles of cattle proved effective for isolating SC from the corresponding muscles in wapiti (Dodson et al. 1987; Burton et al 1999). Wapiti musculature differs from that of cattle by having less connective tissue and subcutaneous fat. Primary cultures of isolated wapiti required numerous washing steps to remove contaminating tissue debris.

Substratum Optimization

The basis for selecting PSG and FN as substrata for SC culture studies has been established (Dodson et al. 1990). In the wapiti system, both PSG and FN at all concentrations supported 100% attachment of wapiti SC while maintaining cell viability (Fig. 1, $P > 0.05$). Pig skin gelatin in conjunction with FBS induced cell proliferation at less than 24 h. Uncoated plastic cultureware and Matrigel® allowed 100% attachment of cells to occur in HS and FBS, although cell morphology and cell health, as indicated by the proportion of attached cells, began to deteriorate after 24 h ($P > 0.05$). Unlike other SC systems in which cultured SC attach and grow differently on specific substrata (Dodson et al. 1990; Venateswaren et al. 1995), wapiti SC growth was consistent on a number of substratum types. These findings suggest that both PSG and FN support wapiti-derived SC attachment. Because it was cost effective and also allowed 100% attachment, 0.02% PSG (0.1 g 500 mL⁻¹) was chosen for use in all subsequent experiments.

Media Characterization

The objective of the proliferation study was to optimize media conditions that facilitate the proliferation of wapiti-derived SC in vitro. Media supplemented with HS and FBS are commonly used to support proliferation in SC systems (Dodson et al 1986, 1987; McFarland et al. 1988; Doumit et al. 1992). Our proliferation experimental findings suggest that DMEM + 10% HS is a good medium to produce proliferation of wapiti-derived SC (Fig. 2). DMEM + 10% HS, DMEM + 15% HS and DMEM + 15% FBS supported the highest levels of growth at 120 h with no significant differences in total cell numbers ($P > 0.05$; Table 1). DMEM +

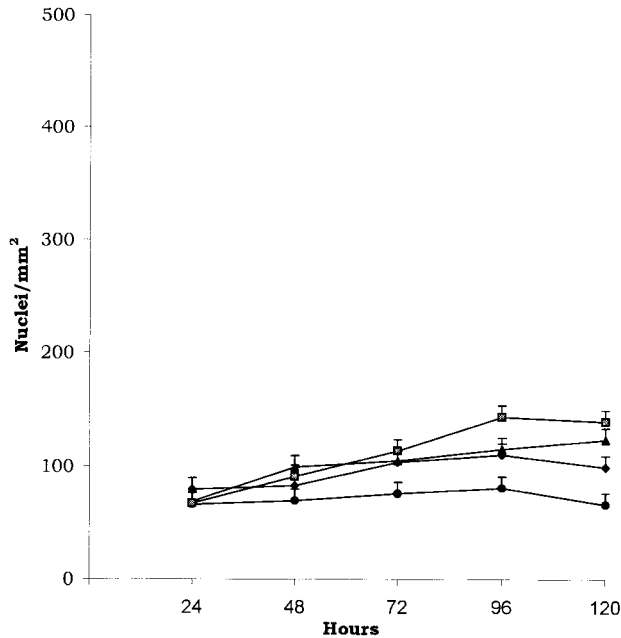


Fig. 3. Effects of serum reduction on wapiti SC growth and differentiation. Cells were plated at 100 nuclei mm^{-2} into 15-mm-diameter culture wells in DMEM + 10% HS. 24 h later cultures were washed with sterile PBS and treatments of DMEM containing 1% (●-●), 2% (◆-◆), 3% (▲-▲) or 4% (■-■) HS were added. At 120 h, cultures were fixed stained and evaluated for SC proliferation and differentiation dynamics. The graph indicates that cell numbers were relatively stable during a 120-h period, with little cell growth or loss and no evident fusion. Each observation represents the sum of six treatment wells (\bar{x} = SEM); 10 randomly chosen microscopic fields were evaluated per well.

2% FBS maintained the total cell numbers of the initial plating density, but DMEM + 2% HS and DMEM + 2% SS demonstrated some cell loss ($P > 0.05$). In DMEM + 2% HS, this cell loss was not as dramatic as that seen in the DMEM + 2% SS treatments. DMEM + 10% SS and DMEM + 15% SS maintained cells at initial plating densities ($P > 0.05$) but did not maintain cell viability. Based on these data, DMEM + 10% HS was selected for use in subsequent experimentation.

A number of experiments were designed to examine differentiation dynamics of wapiti-derived SC. Satellite cells differentiation has been achieved through serum reduction methods (Dodson et al. 1987), growth factor regulation (Dodson and Mathison 1988) and defined media formulations (Erikson et al. 1998). In the present study, the first experiment used four HS concentrations (DMEM + 1% HS, DMEM + 2% HS, DMEM + 3% HS, and DMEM + 4% HS) to determine if any treatment could facilitate wapiti-derived SC differentiation. Cultures were plated at high density (100 nuclei mm^{-2}) to minimize time spent in the proliferation stage (Fig. 3). Results suggested ($P > 0.05$) that none of the treatments induced wapiti SC differentiation. The low percentage of serum treatments did maintain total cell numbers of wapiti-derived SC. This finding is unique, considering

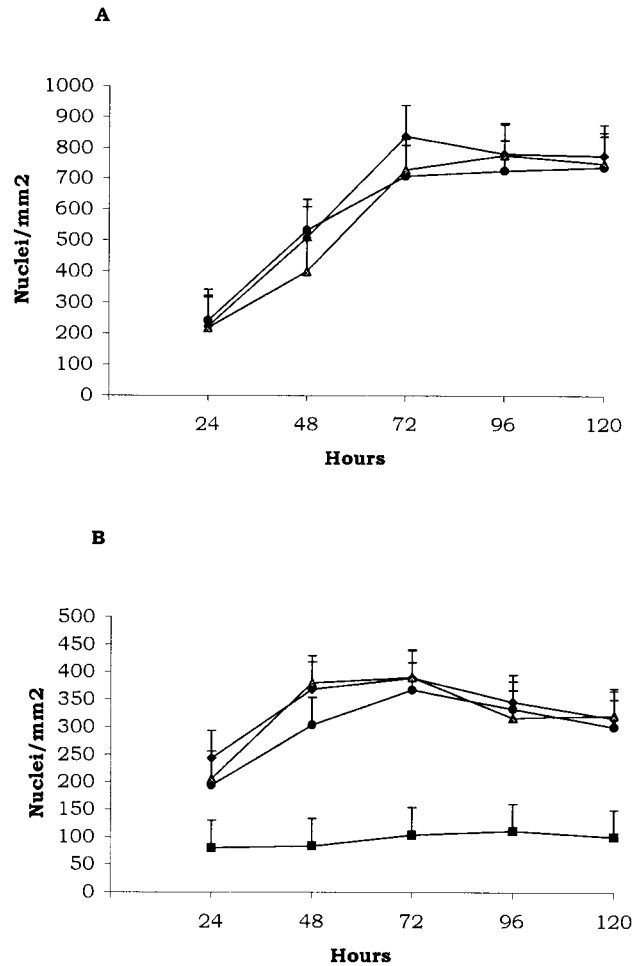


Fig. 4. Effects of insulin on wapiti SC proliferation and differentiation. Cells were plated at 100 nuclei mm^{-2} into 16-mm-diameter culture wells coated with 0.02% PSG in DMEM + 10% HS. 24 h later cultures were washed with sterile PBS and treatments of DMEM containing (A) 10% HS + 0.1 μM (●-●) insulin, 0.5 μM (▲-▲) insulin or 1 μM (◆-◆) insulin or (B) 2% HS + 0.1 μM (●-●) insulin, 0.5 μM (▲-▲) insulin or 1 μM (◆-◆) insulin. In (B) (■-■) represents the 2% HS control. At 120 h, cultures were fixed, stained and evaluated for SC proliferation and differentiation dynamics. Only proliferation was observed. Each observation represents the sum of six treatment wells (\bar{x} = SEM); 10 randomly chosen microscopic fields were evaluated per well.

that many SC systems have demonstrated that a basal media containing 1 or 2% serum promotes differentiation (Dodson et al 1990; Doumit et al. 1992). All treatments experienced some cell loss during the first 24 h of treatment ($P > 0.05$), but DMEM + 1% HS was the only treatment that did not show some level of cell recovery over the next 96 h ($P < 0.05$). Total cells numbers were maintained ($P > 0.05$) in DMEM + 2%, DMEM + 3% and DMEM + 4% HS at the end of the 120-h test period. Cell viability was maintained with all treatments.

Table 2. Mean (\pm SE) of the proliferation (nuclei mm^{-2}) and differentiation (% fusion) characteristics of wapiti SC in four defined media (ITT, ITT-CF, ODM, ODM-Modified) on two substratum types PSG and FN, after 120 h in culture

	Proliferation				Differentiation	
	ITT	ITT-CF	ODM	ODM-Mod	ITT	ITT-CF
PSG	253 \pm 11 ^z	351.5 \pm 25.5	286.5 \pm 13 ^z	271 \pm 23	0.9%	2.9%
FN	240.5 \pm 11 ^z	339 \pm 13	274 \pm 13 ^z	258.5 \pm 23	0.6%	1%

^zSC display different proliferation properties when exposed to ITT or ODM defined media ($P < 0.05$).

Insulin has been shown to exert differentiative effects on SC, presumably through interaction with insulin-like growth factor-I (IGF-I) receptors (Straus 1989). Insulin can mimic the effects of IGF-I (Webster et al. 1996a, 1999), which in turn stimulates differentiation of SC (Gospodarowicz et al. 1976; Doumit and Merkel 1991; McFarland et al. 1993). In order to determine the effects of insulin on wapiti SC, three levels of insulin (0.1 μM , 0.5 μM and 1 μM) were added to DMEM + 2% HS and DMEM + 10% HS at 24 h post plating. Cells were plated at high densities (100 nuclei mm^{-2}) to reduce time spent in the proliferation phase (Fig. 4A and B). No level of insulin induced differentiation in wapiti SC, but all levels induced SC proliferation ($P > 0.05$). The proliferation effects of insulin have been demonstrated in previous experiments with sheep SC (Dodson and Mathison 1998).

In the wapiti system, SC did not preferentially differentiate to form myotubes when exposed to insulin. Instead, total wapiti SC numbers increased substantially. There was no evidence to suggest that the increasing levels of insulin tested affected SC activity differently. Insulin at 10^{-5} M is a supraphysiologic level that interacts with the type-I insulin-like growth factor (IGF) receptors on rat-derived SC (Dodson et al. 1985). The levels tested for wapiti-derived SC were less than 10^{-5} M, which may not constitute saturation levels for wapiti. Ruminant species may differ in the ability of type-I and insulin receptors to promote specific growth responses in SC.

The next experiment assessed the ability of four defined media (ITT, ITT-CF, ODM and ODM-Mod) to induce fusion (Table 2). Cells were plated at high density (100 nuclei mm^{-2}) on two substrata (PSG and FN) to determine if the effects of the defined media could be influenced by different substrata. Specific media/substrata combinations can have varying effects on SC activity (Dodson et al. 1990). Although total cell numbers differed between defined media treatments, total cell numbers did not respond to substrata ($P > 0.05$). ITT-CF induced greater proliferation of wapiti SC than the other three media types ($P < 0.05$). ODM and ODM-Mod produced a slight increase in cell numbers over ITT ($P < 0.05$). During the course of the experiment, SC numbers in the ODM and ODM-Mod reached a plateau at about 24 h and remained stable through 120 h. Cell numbers continued to increase in ITT and ITT-CF media through the 120-h test period.

Only ITT and ITT-CF induced fusion of wapiti SC (Table 2, $P < 0.05$). The highest percent fusion (3%, $P < 0.05$) displayed by wapiti SC exposed to ITT-CF was considerably lower than the fusion percentage of sheep (35–80%; Dodson et al. 1987; Vierck et al. 1995), turkey (80–90%;

McFarland et al. 1991), horse (20–60%; Erickson et al. 1998) and fish (60–90%; Mulvaney and Cyrino 1995) primary SC cultures exposed to differentiative treatments. The reason ITT and ITT-CF promoted some fusion, whereas other differentiation procedures did not, is not known. However, there are several possibilities to explain the reduced fusion seen in the present studies.

For many SC culture systems, a step is added to isolation procedure called a pre-plating step. This step allows fibroblasts to attach to the pre-plate and be removed from the initial suspension of cells, thereby enriching the remaining SC population. However, as our laboratory has shown that the pre-plating step is only required for rodent-derived satellite cell preparations, and not any of the meat animal SC systems, we did not use a pre-plating step for these experiments. The low fusion observed in the present studies could be due to the lack of a pre-plating step.

Perhaps there are components with the serum types tested that suppress SC differentiation activity. Another possibility is that the insulin levels in the defined media ITT and ITT-CF were adequate to initiate wapiti SC differentiation but were not high enough to maintain this activity. Also, differences in the formulation of defined media could induce or suppress differentiation. Some components, such as dexamethasone, are present in ODM, but not in ITT or ITT-CF and could suppress differentiative activity of wapiti SC. The opposite could be true of the component triiodothyronine (T_3), which is unique to ITT and ITT-CF, and stimulated differentiation in quail SC (Marchel et al. 1993). Future studies should address the mechanisms behind the fusion effects of ITT and ITT-CF on wapiti SC.

Differences were also seen in the morphology of wapiti SC in response to different media, but not to substrata. Wapiti SC exposed to ODM and ODM-Mod appeared longer and thinner than wapiti SC exposed to ITT and ITT-CF. Also, there were morphological differences in the myotubes produced. Satellite cells exposed to ITT-CF produced myotubes that were larger and incorporated more myonuclei than those produced in ITT, which were shorter and contained at maximum a total of 12 myonuclei.

CONCLUSIONS

Wapiti SC can be isolated and grown in culture. Proliferation and differentiative effects associated with SC activity can also be induced in cultured wapiti SC. As in other SC systems, these data support the concept that SC influenced wapiti myogenesis. However, the wapiti system displays some differences from other SC culture systems. Wapiti SC appear unique in that basal media containing low

percentages of serum can maintain total cell numbers instead of inducing differentiation. Also, wapiti SC were observed to differentiate in culture through the use of the defined media ITT-CF. Because each animal-derived SC system developed displayed some variability in culture, knowledge of how SC participate in muscle growth is incomplete. Satellite cells research, paired with the holistic/development research of the wildlife biologist, could be used to further our understanding of how skeletal muscle growth occurs within wild animals.

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