

ISSN 1682-8356  
ansinet.org/ijps



INTERNATIONAL JOURNAL OF  
**POULTRY SCIENCE**

**ANSI***net*

308 Lasani Town, Sargodha Road, Faisalabad - Pakistan  
Mob: +92 300 3008585, Fax: +92 41 8815544  
E-mail: editorijps@gmail.com

## Muscle Development in the Late Embryonic and Early Post-Hatch Poult

D.T. Moore, P.R. Ferket and P.E. Mozdziak

Department of Poultry Science, North Carolina State University, Campus Box 7608,  
Scott Hall, Raleigh, NC, 27695, USA  
E-mail: pemozdzi@unity.ncsu.edu

---

**Abstract:** Satellite cells are mitotically active cells in skeletal muscle that contribute new nuclei to growing myofibers. The objective of this experiment was to determine satellite cell mitotic activity in turkey embryos, early post-hatch poults, and 1 week-old poults. All poults were fed a standard corn and soybean meal based starter diet throughout the experiment. 5-bromo-2'deoxyuridine (BrdU) was injected intra-abdominally into all poults and embryos. *Pectoralis thoracicus* were harvested two hours post-injection to determine mitotically active satellite cells. Samples were taken at 25 days of incubation (25E), day-of-hatch, 1 day-of-age, and 1 wk-of -age (n = 10 for all groups). BrdU immunohistochemistry along with computer-based image analysis was used to identify the mitotically active satellite cells. Satellite cell mitotic activity was lower ( $P < 0.05$ ) at 25E compared to day of hatch. Furthermore, there was an age-related decrease ( $P < 0.05$ ) in satellite cell mitotic activity between 1 day posthatch to one week-of-age. The low satellite cell mitotic activity at 25E suggests that late embryonic development may be a developmental period to target increasing satellite cell mitotic activity. Furthermore, the normally high satellite cell mitotic activity immediately post-hatch suggests that the early post-hatch period is also an important target for nutritional manipulations aimed at improving skeletal muscle growth and meat yield.

**Key words:** BrdU, myoblast, satellite cell, myonuclei, myofiber, avian

---

### Introduction

Embryological development of the muscle begins with the fusion of mononucleated myoblasts to form myotubes, which mature into myofibers (Schultz and McCormick, 1994). Feldman and Stockman (1992) showed that during avian development a distinct population of satellite cells is present at the midfetal stages of development, and by late embryogenesis myoblasts found in the chicken embryo are predominantly adult myoblasts (Hartley *et al.*, 1992). Once the bird is hatched and myofibers are formed, normal skeletal muscle growth occurs through an increase in myofiber size, and not an increase in myofiber number (Remignon *et al.*, 1995). Myofiber number does not increase during normal postnatal growth because myonuclei are postmitotic and cannot synthesize DNA (Stockdale and Holtzer, 1961). An increase in myofiber size in postnatal vertebrates is directly related to an increase in myonuclei (Allen *et al.*, 1979). An external cell source must donate nuclei due to the mature myofibers inability to divide. The external source for new myofiber DNA is the satellite cell.

The satellite cell is located beneath the basal lamina of the myofiber, and it is found throughout the length of the myofiber (Mauro, 1961; Campion, 1984). Recently, it has been suggested that muscle satellite cells exhibit multi potential mesenchymal stem cell activity (Asakura *et al.*, 2001). Moss and Leblond (1971) showed that the majority of the mitotically active satellite cells were

incorporated into the myofiber immediately following mitosis in postnatal rats. Myonuclear accretion is regulated by the surrounding myofibers (Bishcoff, 1990; Mozdziak *et al.*, 1998) suggesting stress to the myofiber may increase satellite cell mitotic activity. Animal agriculture may be able to increase muscle size in turkeys by manipulating satellite cell nuclear donation; however, target-ages for the poult must be identified due to age-related changes in satellite cell mitotic activity. Moss and Leblond (1971) showed that satellite cell nuclei are incorporated into the myofiber in the young postnatal rat. Cardasis and Cooper (1975) indicate that satellite cells are first associated with myofibers at 19 days of gestation in the mouse, and satellite cell activity decreases from 32% to 6% from birth to adulthood. The decreases in satellite cells in mice came between 2 weeks of age and 4 weeks of age (Cardasis and Cooper, 1975). Similar results have been reported in turkeys. Mozdziak *et al.* (1994) showed an age-related decrease in satellite cell mitotic activity from 3 to 9 weeks of age in the turkey. However, from 9 to 26 weeks of age myofiber growth in turkeys occurs mainly through an increase in the cytoplasmic to myonucleus ratio (DNA unit size; Mozdziak *et al.*, 1994). Short-term reductions in satellite cell mitotic activity early in life lead to a reduction in muscle size at maturity (Mozdziak *et al.*, 1997; Mozdziak *et al.*, 2000). Overall, it appears that early postnatal muscle development is the most crucial time period for satellite cell mitotic activity.

In the poultry industry, a delay between hatching and placement of the birds in the brooder house is a common management practice. As described previously, the immediate post-hatch period may be a very important developmental window for myonuclear accretion, and it has been shown that delayed placement results in a lower meat yield at market age (Halevy *et al.*, 2000). Hager and Beane (1983) held broiler chicks for varying lengths of time in the incubator to simulate holding times in the industry. Chicks that were held in the incubator for 36 hours post-hatch were 10.3% lighter than chicks that were removed immediately from the incubator. The difference in body weights remained statistically significant at 4 weeks of age (Hager and Beane, 1983; Halevy *et al.*, 2000). It has also been shown that turkey poults held in the incubator for 48 hours post-hatch face an energy shortage resulting in differing body composition than control birds immediately removed from the incubator (Pinchasov and Noy, 1993). Further complicating the lives of poults held in the incubator, additional heat stress may increase mortality during the holding period (Pinchasov and Noy, 1993). Nir and Levanon (1993) found that male broiler chicks held in the incubator for 24 and 48 hours exhibited lower body weights compared to controls. Male broiler chicks with a 24 hour holding time exhibit lower yolk sac content, lower carcass weight at 7 weeks of age, and had increased mortality 21 to 42 days after placement (Vieira and Moran, 1999a). These results suggest a prolonged holding time, as is practiced for practical reasons in the industry, has a definite negative impact on young birds. Other studies involving early post-hatch feed deprivation have also shown decreased body weights and reduced meat yield at market age (Noy and Sklan, 1999b; Vieira and Moran, 1999b). Finally, Mozdziak *et al.* (2002a) observed a larger ratio of apoptotic myonuclei compared to total myonuclei for immediately starved post-hatched chickens when compared to fed controls. The results of delayed placement time of poults may negatively impact satellite cell mitotic activity due to the window of time the satellite cell is normally mitotically active in the young bird. As stated previously, a negative impact on satellite cell mitotic activity (i.e. feed deprivation) at a young age decreases adult muscle weight and the animal may never achieve its full genetic potential for growth (Halevy *et al.*, 2000). Re-feeding after early post-hatch feed-deprivation does not compensate for the missed nutrients, and negatively impacts satellite cell mitotic activity (Mozdziak *et al.*, 2002b). Breast muscle RNA and protein content were also reduced in an immediately starved post-hatch bird following re-feeding (Yaman *et al.*, 2000). Yaman *et al.* (2000) also suggests that re-feeding does not alleviate the effects of early-post hatch feed deprivation. Immediate feeding post-hatch has been shown to increase body weights of birds when

compared to poults with delayed access to feed (Noy and Sklan, 1999a). The objective of these experiments was to determine satellite cell mitotic activity in the turkey embryo, the early post-hatch turkey poult, and the 1-week-old poult.

## Materials and Methods

**Turkeys:** Hybrid turkey eggs were obtained from a commercial hatchery and placed in an incubator at the North Carolina State University hatching facility. Immediately following hatching, poults were placed in floor pens and fed a standard corn and soybean meal starter diet. All birds were weighed at hatch and at seven days of age. Ten birds at 25E, day of hatch, 1-day-old, and 1-week-old were randomly selected from the population of poults and administered with 5-Bromo-2'-deoxyuridine (BrdU; 10mg/ml; 1ml/100g of bird weight), a thymidine analog. All embryos and poults were injected with BrdU intra-peritoneally. The eggs were candled to determine the location of the abdomen in the 25E embryos. Once the abdomen was located, a small hole was incised in the shell directly above the site of the intended BrdU injection. Once injected, the holes were sealed with scotch tape and the eggs were returned to the incubator until sampling. The BrdU was administered using a 22-gauge hypodermic needle fitted to a syringe for all embryos and poults. After the BrdU injection, two hours were allowed for the BrdU to be incorporated into the nuclei entering the S-phase of the cell cycle. The poults and embryos were killed at the end of two the hours by cervical dislocation. All procedures involving animals were approved by the North Carolina State University Animal Care and Use Committee.

**Immunohistochemistry:** The *Pectoralis thoracicus* was harvested and fixed with Carnoy's solution (60% ethanol, 30% chloroform, and 10% glacial acetic acid) overnight in 20ml scintillation vials. The next morning the tissues were dehydrated, washed, and embedded in paraffin. Eight micron thick sections were cut on a microtome and adhered to glass slides. Tissues were de-waxed and hydrated. Following hydration of the tissues, 0.07N NaOH was added to the slides for three minutes to denature DNA. Phosphate Buffered Saline (PBS-pH 7.4) was used to neutralize the NaOH. The primary monoclonal antibody Anti-BrdU (Becton Dickinson, Mountain View, CA) diluted 1:20 in PBS containing 0.5% tween-20 and 0.5% bovine serum albumin was added to the sections and incubated for two hours in a humidified chamber. After the tissue section was blocked with PBS containing 10% goat serum and 0.5%tween-20 for fifteen minutes to minimize background staining; the secondary antibody was added to the sections for two hours in a humidified chamber to detect the BrdU labeled nuclei. The secondary antibody

Table 1: Body weights in grams at hatch and one week of age

	Hatch (g)	One Week of Age (g)
Body Weights (g)	55.8 ± 0.4 <sup>b</sup>	134.8 ± 2 <sup>a</sup>

<sup>a,b</sup>Values within columns without a common superscript are significantly different ( $P < 0.05$ ). Values are means + SE.

was goat anti-mouse IgG conjugated to fluorescein-isothiocyanate (FITC), which was diluted 1:50 with PBS containing 10% goat serum and 0.5% tween-20. Propidium iodide (50 µg/ml in PBS) was added to the sections for 20 minutes to label all nuclei. Finally, the sections were placed in mounting media [75% (vol/vol), 75mM KCL, 10mM tris (hydroxymethyl) aminomethane, 2mM MgCl, 2mM ethylene glycol-bis (β-amino-ethyl ether)-N,N,N',N'- tetraacetic acid, 1mM NaN<sub>3</sub>, pH 8.5, 1mg/mlp-phenylenediamine] and a cover slip was sealed using nail polish.

**Image analysis:** A Leica DMR (Leica Microsystems, Bannockburn, IL) microscope with epifluorescence illumination was used to observe the tissue sections. All nuclei were observed with a propidium iodide (PI) filter set (Omega Optical, Brattleboro, VT) and the BrdU labeled nuclei were observed with a FITC filter set. A Spot-RT CCD (Diagnostic Instruments Inc., Sterling Heights, MI) camera was used to capture the images of nuclei visualized by the FITC and PI filter sets. The FITC and PI labeled nuclei were counted using the Image-Pro Plus software (Media Cybernetics, Silver Spring MD). The number of BrdU labeled nuclei per 1000 PI labeled nuclei was used to calculate an index of satellite cell mitotic activity. The criteria for completing nuclear analysis was counting at least 1000 PI labeled nuclei. The area of muscle section containing the count of propidium iodide labeled nuclei was also determined. The purpose of collecting the muscle section area was to express the number of PI labeled nuclei in relation to the cross-sectional area of the muscle. Image-Pro Plus software was also used to determine myofiber cross-sectional area for at least 100 myofibers per muscle sample.

**Statistics:** General Linear Models procedure of SAS (SAS Institute, 1985) was used to perform a one-way analysis of variance to determine age effects on body weight, the number of PI labeled nuclei per muscle area, myofiber cross-sectional area, and satellite cell mitotic activity. Means were separated using least significant differences (Zar, 1999).

## Results

**Growth:** Body weights significantly ( $P < 0.05$ ) increased between hatch and 1-week of age (Table 1). Myofiber cross-sectional areas also significantly ( $P < 0.05$ )

increased between of age 25E and 1-week of age.

**Satellite Cell Mitotic Activity and Number of Total Nuclei:** Satellite cell mitotic activity was lowest ( $P < 0.05$ ) on 25E and the highest ( $P < 0.05$ ) on one day of age. Subsequently, satellite cell mitotic activity was lower ( $P < 0.05$ ) in 1 week-old poults compared to 1-day-old poults (Table 2). Also nuclei per unit area measured was lower ( $P < 0.05$ ) at one week of age compared to day-old poults suggesting an age-related increase in DNA unit size (Table 2).

## Discussion

A common practice in the poultry industry is holding poults in the incubators for 48 to 72 hours post-hatch. The practice of holding poults has been implemented out of the necessity to place large numbers of turkeys in poultry houses at one time. However, the result of holding poults without food or water post-hatch can be detrimental because it decreases satellite cell proliferation and overall skeletal muscle growth (Halevy *et al.*, 2000). Growth retardation has been seen in male broiler chicks that were held in the incubator for 24 and 48 hours resulting in a decrease in body weight at market age when compared to chickens removed immediately from the incubator (Nir and Levanon, 1993). Holding time in the incubator has also been attributed to weight loss in the poult and chick that was still present at market age (Hager and Beane, 1983; Pinchasov and Noy, 1993; Halevy *et al.*, 2000). Chicks held in the incubator also experience a decreased yolk nutrient content and increased mortality (Vieira and Moran, 1999a), which suggests a nutrient deficit in the post-hatch bird with delayed access to feed and water. Feed deprivation trials have also been performed to simulate increased holding time in the incubator and similar results to the holding trials were attained (Noy and Sklan, 1999b; Vieira and Moran, 1999b). It is likely that a developmental window in muscle development is being missed due to delayed access to feed and water because it has been shown in other models that when muscle is stressed during this time period the muscle never recovers (Mozdziak *et al.*, 1997; Mozdziak *et al.*, 2000).

One indicator of muscle development is satellite cell mitotic activity. Mozdziak *et al.* (1997) suggests that satellite cell mitotic activity and subsequent myofiber nuclear accretion is a major factor determining turkey muscle growth potential. In the turkey, there is an age-related decrease in satellite cell mitotic activity (Mozdziak *et al.*, 1994). Research also indicates a similar age-related decline in satellite cell mitotic activity in other juvenile animals (Allen *et al.*, 1979; Moss and Leblond, 1971; Cardasis and Cooper, 1975). Therefore, it is not surprising that satellite cell mitotic activity was lower in 1-week-old poults compared to day-old poults. However,

Table 2: Myofiber cross-sectional area, satellite cell mitotic activity and nuclei per unit area

Age	Myofiber CSA <sup>A</sup> ( $\mu\text{m}^2$ )	Satellite Cell Mitotic Activity <sup>B</sup>	Nuclei per $\mu\text{m}^2$ <sup>C</sup>	Sample size
25E	18.7 $\pm$ 1.44 <sup>b</sup>	0.014 $\pm$ 0.0018 <sup>d</sup>	0.015 $\pm$ 0.0002 <sup>a</sup>	10
Day of Hatch	11.3 $\pm$ 0.96 <sup>c</sup>	0.078 $\pm$ 0.016 <sup>b</sup>	0.014 $\pm$ 0.0003 <sup>b</sup>	10
1 day old	8.6 $\pm$ 1.90 <sup>c</sup>	0.137 $\pm$ 0.013 <sup>a</sup>	0.015 $\pm$ 0.0008 <sup>a</sup>	10
1 wk old	79.8 $\pm$ 5.66 <sup>a</sup>	0.047 $\pm$ 0.006 <sup>c</sup>	0.008 $\pm$ 0.0003 <sup>c</sup>	10

<sup>A</sup>CSA = cross-sectional area. <sup>B</sup>Satellite cell mitotic activity is expressed as the number of BrdU-fluorescein, isothiocyanate labeled nuclei per 1000 propidium iodide labeled nuclei. <sup>C</sup>Nuclei per  $\mu\text{m}^2$  is the number of propidium iodide labeled nuclei per  $\mu\text{m}^2$  of tissue section. <sup>a,b,c,d</sup>Values within columns without a common superscript are significantly different ( $P < 0.05$ ). Values are means  $\pm$  SE.

it is interesting that satellite cell mitotic activity was significantly higher at day-of-hatch and 1 day-of-age compared to the 25E embryo. The post-hatch age-related decrease in satellite cell mitotic activity suggest that the greatest influence of muscle development in the poult is the first few days post-hatch, which is the exact time frame that poults are normally without access to feed and water. It is therefore evident that satellite cell mitotic activity is adversely affected by extended holding time in the incubator. When young animals have a negative stress on the muscle, satellite cell mitotic activity decreases (Mozdziak *et al.*, 1998; Mozdziak *et al.*, 2000). A temporary reduction in satellite cell mitotic activity (myonuclear accretion) at a young age results in a decreased weight of the adult muscle (Mozdziak *et al.*, 2000) suggesting that holding poults in the incubator without food or water will not only decrease satellite cell mitotic activity, but will also result in decreased muscle weight at market age.

The results of this study suggests that the immediate post-hatch period may be the most critical post-hatch time for satellite cell mitotic activity as shown by a significantly higher satellite cell proliferation in the day-old-poult when compared to a seven-day-old poult. The findings of the present study also suggest that satellite cell mitotic activity is significantly lower ( $P < 0.05$ ) at 25E than at hatch suggesting that 25E may be a pre-hatch developmental window that may be targeted by late embryonic *in ovo* nutritional regimens aimed at improving ultimate meat yield (Tako *et al.*, 2004). It has been previously shown that muscle organizational differences between sexes in turkey embryos are apparent by 25E and satellite cell proliferation and differentiation differences exist between turkeys selected for different growth rates (Velleman *et al.*, 2000; 2002). These data (Velleman *et al.*, 2000; 2002) support the idea that approximately 25E in the turkey embryo represents a potential developmental target for improving muscle development. The identified embryonic and early post-hatch targets for muscle development in the post-hatch bird may be potentially nutritionally manipulated to improve meat yield, and warrants further investigation.

### Acknowledgements

We thank Pam Jenkins for assistance with the statistical analysis. We also thank Annette Israel, Carol Morris,

Darell McCoy, and Steve Holcutt for technical assistance. We lastly thank Prestage Farms for providing the embryos employed in this study. Support provided in part by funds under project number NC06590 (PEM) and NC06343 (PRF) of North Carolina State University.

### References

- Allen, R.E. and L.K. Boxhorn, 1989. Regulation of skeletal muscle satellite cell proliferation and differentiation by transforming growth factor-beta, insulin-like growth factor 1, and fibroblast growth factor. *J. Cell. Physiol.*, 138: 311-315.
- Allen, R.E., R.A. Merkel and R.B. Young, 1979. Cellular aspects of muscle growth: myogenic cell proliferation. *J. Anim. Sci.* 49: 115-127.
- Asakura, A., M. Komaki and M.A. Rudnicki, 2001. Muscle satellite cells are multipotential stem cells that exhibit myogenic, osteogenic, and adipogenic differentiation. *Diff.* 68: 245-253.
- Bischoff, R., 1990. Interaction between satellite cells and skeletal muscle fibers. *Development*, 109: 943-952.
- Campion, D.R., 1984. The muscle satellite cell: a review. *Int. Rev. Cytol.*, 87: 225-251.
- Cardasis, C.A. and G.W. Cooper, 1975. An analysis of nuclear numbers in individual muscle fibers during differentiation and growth: a satellite cell-muscle fiber growth unit. *J. Exp. Zool.*, 191: 347-351.
- Feldman, J.L. and F.E. Stockman, 1992. Temporal appearance of satellite cells during myogenesis. *Dev. Biol.*, 153: 217-226.
- Hager, J.E. and W.L. Beane, 1983. Posthatch incubation time and early growth of broiler chicks. *Poult. Sci.*, 62: 247-254.
- Halevy, O., A. Geyra, M. Barak, Z. Uni and D. Sklan, 2000. Early posthatch starvation decreases satellite cell proliferation and skeletal muscle growth in chicks. *J. Nutr.*, 130: 858-864.
- Hartley, R.S., E. Bandman and Z. Yablonka-Reuveni, 1992. Skeletal muscle satellite cells appear during late chicken embryogenesis. *Dev. Bio.*, 153: 206-216.
- Mauro, A., 1961. Satellite cells of skeletal muscle fibres. *J. Biophys. Biochem. Cyto.*, 9: 493-495.
- Moss, F.P. and C.P. Leblond, 1971. Satellite cells as the source of nuclei in muscles of growing rats. *Anat. Rec.*, 170: 421-436.

- Mozdziak, P.E., J.J. Evans and D.W. McCoy, 2002a. Early posthatch starvation induces myonuclear apoptosis in chickens. *J. Nutr.*, 132: 901-903.
- Mozdziak, P.E., E. Schultz and R.G. Cassens, 1994. Satellite cell mitotic activity in posthatch turkey skeletal muscle growth. *Poult. Sci.*, 73: 547-555.
- Mozdziak, P.E., E. Schultz and R.G. Cassens, 1997. Myonuclear accretion is a major determinant of avian skeletal muscle growth. *Am. J. Physiol.*, 272: C565-571.
- Mozdziak, P.E., P.M. Pulvermacher and E. Schultz, 2000. Unloading of juvenile muscle results in a reduced muscle size 9 wk after reloading. *J. Appl. Physiol.*, 88: 158-164.
- Mozdziak, P.E., Q. Truong, A. Macius and E. Schultz, 1998. Hindlimb suspension reduces muscle regeneration. *Eur. J. Appl. Physiol.*, 78: 136-140.
- Mozdziak, P.E., T.J. Walsh and D.W. McCoy, 2002b. The effect of early posthatch nutrition on satellite cell mitotic activity. *Poult. Sci.*, 81: 1703-1708.
- Nir, I. and M. Levanon, 1993. Research note: effect of posthatch holding time on performance and on residual yolk and liver composition. *Poult. Sci.*, 72: 1994-1997.
- Noy, Y. and D. Sklan, 1999a. Different types of early feeding and performance in chicks and poults. *J. Appl. Poult. Res.*, 8: 16-24.
- Noy, Y., and D. Sklan, 1999b. Energy utilization in newly hatched chicks. *Poult. Sci.*, 78: 1750-1756.
- Pinchasov, Y. and Y. Noy, 1993. Comparison of post-hatch holding time and subsequent early performance of broiler chicks and turkey poults. *Br. Poult. Sci.*, 34: 111-120.
- Remignon, H., M.F. Gardahaut, G. Marche and F.H. Ricard, 1995. Selection for rapid growth increases the number and the size of muscle fibres without changing their typing in chickens. *J. Mus. Res. Cell. Mot.*, 16: 95-102.
- SAS Institute, 1985. User's Guide: Statistics, Version 5 Edition, SAS Institute, Cary, N.C.
- Schultz, E. and K.M. McCormick, 1994. Skeletal muscle satellite cells. *Rev. Phys. Biochem. Pharm.*, 123: 213-57.
- Stockdale, F.E. and H. Holtzer, 1961. DNA synthesis and myogenesis. *Exp. Cell Res.*, 24: 508-520.
- Tako, E., P.E. Ferket and Z. Uni, 2004. Effects of *in ovo* feeding of carbohydrates and beta-hydroxy-beta-methylbutyrate on the development of chicken intestine. *Poult. Sci.*, 83: 2023-2028.
- Velleman, S.G., C.S. Coy, J.W. Anderson, R.A. Patterson and K.E. Nestor, 2002. Effect of selection for growth rate on embryonic breast muscle development in turkeys. *Poult. Sci.*, 81: 1113-1121.
- Velleman, S.G., X. Liu, K.E. Nestor and D.C. McFarland, 2000. Heterogeneity in growth and differentiation characteristics in male and female satellite cells isolated from turkey lines with different growth rates. *Comp. Biochem. Phys. Part A*, 125: 503-509.
- Vieira, S.L. and E.T. Moran, Jr., 1999a. Effects of delayed placement and used litter on broiler yields. *J. Appl. Poult. Res.*, 8: 75-81.
- Vieira, S.L. and E.T. Moran, Jr, 1999b. Effects of egg origin and chick post-hatch nutrition on broiler live performance and meat yields. *World's Poult. Sci. J.*, 55: 125-142.
- Yaman, M.A., K. Kita and J. Okumura, 2000. Different responses of protein synthesis to refeeding in various muscles of fasted chicks. *Br. Poult. Sci.*, 41: 224-228.
- Zar, J.H., 1999. *Biostatistical Analysis*. Prentice Hall, Upper Saddle River, New Jersey.