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LINKING EMBRYONIC MYOGENESIS TO MEAT QUANTITY AND QUALITY

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The discipline of meat science has classically focused on *ante-mortem* and *post-mortem* handling procedures that effect ultimate meat quality. Furthermore, meat science has also ventured into genetic and physiological factors that impact ultimate meat quality. However, in general, meat scientists have not fully considered the impact of embryonic development nor have they targeted the embryo in strategies aimed at optimizing meat quality. Embryonic development has a profound impact on ultimate meat yield and meat quality because embryonic events program muscle phenotype, muscle growth potential, ultimate muscle size, and muscle metabolic potential. In farm animals, myofiber size, contractile protein composition, and myofiber phenotype have a profound impact on eating quality. During development, gastrulation begins when the blastoderm invaginates to form the endoderm, mesoderm, and the ectoderm. The somites, derived from the mesoderm, are the classically accepted site of myogenesis. The underlying mechanisms governing myogenesis, regulating myofiber number, and determining myofiber phenotype are not yet fully understood. The focus of this manuscript is to review the general embryonic mechanisms governing muscle development and to speculate about potential targets to improve meat quality through embryonic manipulation.

INTRODUCTION

The discipline of classical meat science has tended to focus on the immediate ante-mortem and post-mortem biological mechanisms/pathways that affect appearance, flavor, and tenderness as measures of ultimate meat quality. However, little attention has been placed by meat scientists upon the effect of embryonic influences on ultimate meat quality. Given the emphasis over approximately the last 80 years on optimizing animal management, and the last 60 years of meat science as a distinct discipline, it is likely that completely optimal production systems will soon be in place, and that the information is already available to maximize ultimate meat quality. However, outside of genetic manipulation, the embryo is likely the last target of management systems to maximize meat yield as well as meat quality. As with all animal production strategies, it is important to understand the mechanisms governing muscle development to devise new methodologies aimed at improving meat quality through embryonic management or manipulation. Therefore, this article will review the basic mechanisms of muscle development in the embryo.

EMBRYONIC DEVELOPMENT

The chicken is the classical model for developmental biology because the accessibility of the chick embryo allows embryonic observation and manipulations that are not possible in a mammalian system. The chick embryo is presented as the model system for muscle development because the general mechanism between chick development and mammalian development is essentially the same. Furthermore, the chick embryo has been the classical model for human development for more than a century.

Construction of an avian egg begins with the ovulation of a mature ovum from the ovary. Sperm are stored in the sperm host gland of the female, and fertilization happens when the ovum enters the infundibulum of the oviduct. The fertilized egg enters the magnum, which secretes albumen. Subsequently, the ovum enters the isthmus where the outer and inner shell membranes are deposited to prepare the egg for shell formation in the shell gland. It takes approximately 3.5 to 4 hours for the ovum to move from the infundibulum into the shell gland, where the egg remains for the approximately 20 hours it takes to form the eggshell. The first cleavage division occurs upon entry of the ovum into the shell gland. Over the 20 hours it takes to form an egg shell, cell divisions continue, and at lay, the embryo is at the blastodermal stage and comprises 50,000–60,000 cells on the surface of the yolk mass [Spratt & Haas, 1960]. Although blastodermal cells may express myogenic markers such as the DNA binding protein MyoD, and myogenic commitment may occur for a population of cells within the blastoderm [George-Weinstein *et al.*, 1996; Gerhart *et al.*, 2000; 2004], the classical generally accepted primary site of myogenesis is the somite [Williams *et al.*, 1910; Kaehn *et al.*, 1988; Christ & Ordahl, 1995; Denetclaw *et al.*, 1997; Brand-Saberi & Christ, 2000].

The blastoderm of the freshly laid egg contains an area pellucida, which forms the actual embryo, and the area pellucida is surrounded by the area opaca, which contributes to the extraembryonic membranes. The blastoderm also consists of an upper epiblast, which produces the three germ lay-

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ers (endoderm, mesoderm, and ectoderm). The blastoderm also contains a lower hypoblast that only contributes to the extra-embryonic circulation [Petitte *et al.*, 1997]. The space between the epiblast and the hypoblast is analogous to the mammalian blastocoel.

The next major structural change after epiblast and hypoblast formation is the thickening at the posterior end of the embryo to form the primitive streak. Ingression of endodermal precursors from the epiblast into the blastocoel, and by the migration of cells from the lateral region of the posterior epiblast toward the center of the embryo forms the primitive streak. As cells enter the primitive streak, it elongates toward the anterior portion of the embryo and a depression occurs in the developing embryo forming the primitive groove. At the anterior end of the primitive streak, a regional thickening called Henson's node forms. The center of Henson's node contains a depression called the primitive pit where cells pass into the blastocoel (Figure 1). The primary organizer of chick development is Henson's node because removing Henson's node from a developing embryo results in embryonic structural malformations [Charrier *et al.*, 2005; Stern, 2005]. Eventually, the primitive streak regresses, and it is replaced by the notochord. Formation of mesodermal and endodermal organs occurs simultaneously with neural tube formation. The notochord develops into the vertebral column, and the neural tube develops into the central nervous system. During early embryonic development, the neural tube forms over the notochord from the base of the head into the tail. The paraxial mesoderm separates from the neural tube and forms blocks of cells called somites, as the primitive streak regresses and neural folds form at the center of the embryo [Stern, 1994, 2005; Stern & Hauschka, 1994]. Somites are transient structures that are essential for the pattern formation of muscle, and they are the generally accepted major site of myogenesis [Chevallier *et al.*, 1977; Christ *et al.*, 1977; 1978; Kaehn *et al.*, 1988; Ordahl & LeDourain, 1992; Ordahl *et al.*, 2001].

Upon initial somite formation, the cells that comprise the somite are pluripotent [Aoyama; 1993; Pourquie, 2001]. However, the somite can be partitioned into three subdivisions, the sclerotome, the dermatome, and the myotome. The sclerotome will form the cartilage of the vertebrae and the rib. The dermatome generates the mesenchymal cells that form the connective tissue underlying the dermis [Pourquie, 2001].

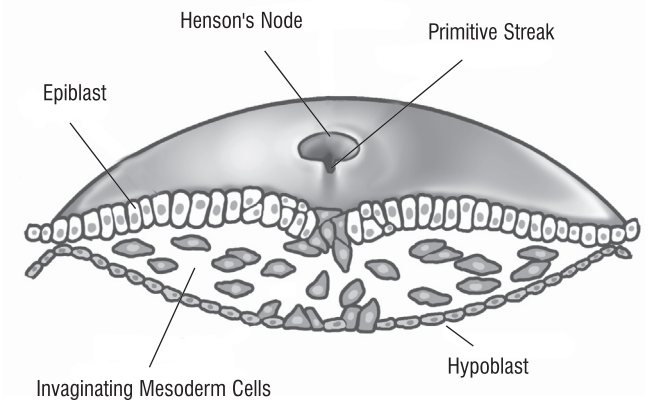

FIGURE 1. Image illustrating the epiblast, the hypoblast, Henson's node, and the invaginating mesodermal cells. Based upon a figure in Langman's Medical Embryology [Sadler, 2005].

The myotome is the portion of the somite where muscle arises, and the myotome of the somite can be subdivided into the epaxial portion and the hypaxial portion [Hawke & Garry, 2001]. The epaxial myotome forms the back muscles whereas the hypaxial myotome forms the muscles of the body wall including the abdominal muscle and limb [Hawke & Garry, 2001; Christ & Brand-Saberi, 2002] and both the epaxial myotome and the hypaxial myotome arise from the dermamyotome in the somite. The hypaxial cells migrate and form the limb bud, but when the receptor for hepatocyte growth factor (c-met) is absent then limb muscles do not form [Bladt *et al.*, 1995] suggesting that there is a complex signaling mechanism governing cell fate in the embryo (Figure 2). It is important for meat scientists to appreciate rudimentary pattern formation of skeletal muscle because the developmental processes determine the ultimate shape and constituents of the final meat product. It has been well established that certain muscles such as the *psoas major* (arising from the epaxial myotome) have more desirable eating characteristics than other muscle such as the rectus abdominus arising from the epaxial myotome. The underlying differences in tenderness and eating quality may be attributed to muscle function and connective tissue content, but it is these underlying embryonic developmental process that dictate muscle location, muscle function, muscle connective tissue composition and ultimately final eating quality.

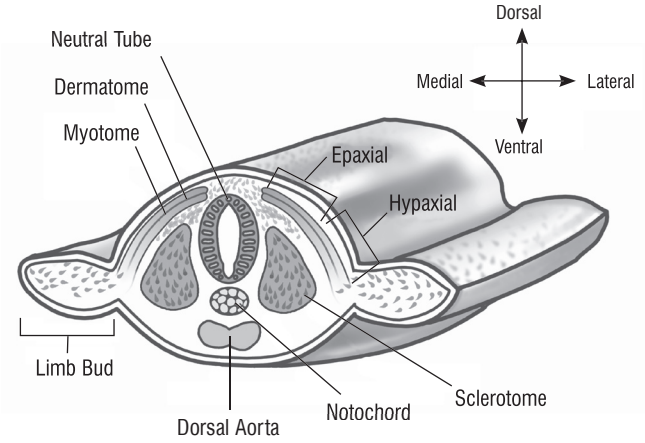

FIGURE 2. Schematic representation of muscle formation during embryonic development. The somite can be divided into the dermatome, the myotome, and the sclerotome. The dermatome develops into the tissue underlying the skin, and the sclerotome develops into the vertebral column. The myotome can be subdivided into a hypaxial region that develops into the back muscles, wheras the hypaxial myotome develops into muscles of the body wall/abdomen and the limbs. Drawing is based on Hawke & Garry [2001].

CELL SPECIFICATION

The somites are generally regarded as the site of myogenesis, but the process of myogenic specification is quite complex. Myogenic specification within the somite appears to be tied to cues from the notochord, dorsal ectoderm, neural tube, and specification is closely tied to the expression of myogenic regulatory factors such as Pax-3 and Pax-7 [Cossu

et al., 1996; Borycki *et al.*, 1995a, b; Wagers & Conboy, 2005] Once a cell becomes a myoblast it migrates to a site of myogenesis, and it begins to enter into terminal differentiation by expressing myogenin [Smith *et al.*, 1994; Andres & Walsh, 1996]. The cells complete terminal differentiation by fusing to form multinucleate myotubes. Once a cell fuses with the myotube it becomes terminally differentiated and it can no longer proliferate. Furthermore, proliferating myoblasts do not express myofibrillar proteins, and once myofibrillar proteins appear cells are terminally differentiated. After myotube formation in mammals, they exclusively exhibit central nuclei, but as the myotubes mature into myofibers the nuclei undertake a peripheral position. The postnatal myofiber is post-mitotic, multinucleate, and scientific dogma suggests that postnatal muscle growth occurs exclusively through an increase in myofiber size without an increase in myofiber number. Therefore, embryonic events limit the growth potential of the animal, and a limiting factor to myofiber growth is the amount of cytoplasm that an individual nucleus can support [Landing *et al.*, 1974]. However, the postnatal increase in muscle size occurs concurrent with the increase in DNA content. Since myofibers are post-mitotic the DNA must come from an exogenous cell source. In skeletal muscle, a population of satellite cells lies outside the myofiber sarcolemma, but within the basal lamina, and they are called satellite cells [Mauro, 1961]. The role of satellite cells during normal muscle growth is to proliferate and donate nuclei to the growing myofiber [Moss & Leblond, 1971]. Furthermore, it has recently been shown the myoblasts and satellite cells arise from the same embryonic lineage [Gros *et al.*, 2005, Relaix *et al.*, 2005].

SATELLITE CELLS

Satellite cells have been classically defined by their position between the sarcolemma and the myofiber basal lamina, and classically satellite cells were thought to be committed to myogenesis, whereas cells within the connective tissue matrix were thought to be incapable of undertaking a myogenic fate. The first evidence of satellite cell heterogeneity was that satellite cells formed large colonies and small colonies when maintained in vitro [Schultz & Lipton, 1982] followed by the discovery that some satellite cells (~80% of the population) represent a rapidly cycling cell population while other satellite cells represent a very slowly dividing population [Schultz, 1996]. Therefore, quiescent and dividing satellite cells are found in post-natal muscle. However, the most intriguing findings that changed the satellite cell field were that cells isolated from skeletal muscle were not all committed to myogenesis [Jackson *et al.*, 1999; Asakura *et al.*, 2001; Mckinney-Freeman *et al.*, 2002]. Subsequently, it was discovered that some cells within the satellite cell population did not express myogenic markers such as MyoD and myogenin, while some cells in the connective tissue compartment did express myogenic markers [Tamaki *et al.*, 2002a, b]. Furthermore, it has been shown that satellite cells can undertake alternative fates to myogenesis, such as adipogenesis [Shefer *et al.*, 2004]. Therefore, the precise relationship, definition, and functional significance of the cells within the satellite cell population and the endomysial connective tissue compartment has become much more controversial over the last several years. The firm conclusion that can be drawn about proliferating muscle or muscle precursor cells is that cells within the satellite cell population can undertake phenotypes other than skeletal muscle, while a subset of cells within the connective tissue compartment can undertake a myogenic fate.

The functional significance of the heterogenous nature of satellite cells and fibroblasts is presently not completely understood, but the implications for meat quality and quantity are obvious. Firstly, if the cell populations can be manipulated prenatally or postnatally to form a desired amount of intramusclar fat to meet current market demands, it will be possible to engineer beef animals with a high fat content to benefit a niche market for high intramusclar fat/high quality meat. Alternatively, if it is possible to direct cells toward lean muscle mass and away from fat, it will be possible to optimize lean meat accretion as well as engineer a product with the low-fat content for the health conscious consumer.

Another obvious effect of embryonic development on meat quality is the establishment of myofiber type diversity. Red or slow myofibers are characterized by high myoglobin content, slow contraction speeds, oxidative metabolism, and they are specialized to meet long-term demands on the muscle. In contrast, white or fast myofibers are characterized by low myoglobin, fast contraction speeds, glycolytic metabolism, and they are specialized to meet short-term needs. It appears that myofiber type is programmed in the embryo because myofiber type diversity is established early in development, but innervation also appears to impact myofiber phenotype [DiMario & Stockdale, 1997]. Furthermore, satellite cells remain faithful to the phenotype of their host myofiber when they are cultured *in vitro* [Rosenblatt *et al.*, 1996]. It has been proposed that the myogenic regulatory factors MyoD and myogenin may be causative factors governing myofiber phenotype because MyoD has been associated with fast myofibers and myogenin has been associated with slow myofibers [Voytik *et al.*, 1993 Hughes *et al.*, 1993]. It appears that there is not a simple relationship between myofiber phenotype and myogenin/myoD expression [Jacobs-El *et al.*, 1995; Kraus & Pette, 1997]. However, it may be possible to alter the eating characteristics of an animal through postnatal manipulation because over-expressing myogenin has altered the oxidatative capacity/phenotypic characteristics of myofibers [Hughes *et al.*, 1999; Ekmark *et al.*, 2003]. It may also be possible to engineer muscle with a specific myofiber phenotype by directing cells of a certain lineage toward specific muscle fates, but muscle phenotype is partially dictated by anatomical location and functional demand as well as a genetic prediposition to a certain phenotype.

In summary, the emphasis of this manuscript is to review the key aspects of embryonic muscle development to remind meat scientists that the embryonic system predisposes an animal to generate a carcass with certain eating characteristics. Production systems and *post-mortem* handling procedures are likely nearing full optimization leaving the only targets for optimizing meat yield to be genetics and embryonic development. Genetic manipulations will likely always take a significant amount of time because genetic optimization occurs over several generations whereas manipulating embryonic development to meet immediate production targets can occur on demand.

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REFERENCES

1. Andres V., Walsh K., Myogenin expression, cell cycle withdrawal, and phenotypic differentiation are temporally separable events that precede cell fusion upon myogenesis. J. Cell Biol., 1996, 132, 657–666.
2. Aoyama H., Developmental plasticity of the prospective dermatome and the prospective sclerotome region of an avian somite. Develop. Growth Diff., 1993, 35, 507–519.
3. Asakura A., Komaki M., Rudnicki M.A., Muscle satellite cells are multipotential stem cells that exhibit myogenic, osteogenic, and adipogenic differentiation. Differentiation, 2001, 68, 245–253.
4. Bladt F., Riethmacher D., Isenmann S., Aguzzi A., Birchmeier C., Essential role for the c-met receptor in the migration of myogenic precursor cells into the limb bud. Nature, 1995, 376, 768–771.
5. Borycki A.G., Brunk B., Tajbakshk S., Buckingham M., Chiang C., Emerson C.P., Shh is essential for the determination of the epaxial muscle lineage. Dev. Biol., 1995a, 210, 227–227.
6. Borycki A.G., Li J., Jin F.Z., Emerson C.P., Epstein J.A., Pax3 functions in cell survival and in pax7 regulation. Development, 1995b, 126,1665–1674.
7. Brand-Saberi B., Christ B., Evolution and development of distinct cell lineages derived from somites. Curr. Top. Dev. Biol., 2000, 48, 1–42.
8. Charrier J.B., Catala M., Lapointe F., Le Douarin N., Teillet M.A., Cellular dynamics and molecular control of the development of organizer-derived cells in quail-chick chimeras. Inter. J. Dev. Bio., 2005, 49, 181–191.
9. Chevallier A., Kieny M., Mauger A., Limb-somite relationship – origin of limb musculature. J Embry. Exper. Morph., 1977, 41, 245–258.
10. Christ B., Jacob H.J., Jacob M., Experimental–analysis of origin of wing musculature in avian embryos. Anat. Embry., 1977, 150, 171–186.
11. Christ B., Jacob H.J., Jacob M., Experimental-study on relative distribution of somitic and somatic plate mesoderm to abdominal-wall of avian embryos. Experientia, 1978, 34, 241–242.
12. Christ B., Ordahl C.P., Early stages of chick somite development. Anat. Embry., 1995, 191, 381–396.
13. Christ, B., Brand-Saberi B., Limb muscle development. Int. J. Dev. Biol., 2002, 46, 905–914.
14. Cossu G., Kelly R., Tajbakhsh S., DiDonna S., Vivarelli E., Buckingham M., Activation of different myogenic pathways: Myf-5 is induced by the neural tube and MyoD by the dorsal ectoderm in mouse paraxial mesoderm. Development, 1996, 122, 429–437.
15. Denetclaw W.F., Christ B., Ordahl C.P., Location and growth of epaxial myotome precursor cells. Development, 1997, 124, 1601–1610.
16. DiMario J.X., Stockdale F.E., Both myoblast lineage and innervation determine fiber type and are required for expression of the slow myosin heavy chain 2 gene., Dev. Biol., 1997, 188, 167–180.
17. Ekmark M., Gronevik E., Schjerling P., Gundersen K., Myogenin induces higher oxidative capacity in pre-existing mouse muscle fibres after somatic DNA transfer. J. Physiol. – London, 2003, 548, 259–269.
18. George-Weinstein M., Gerhart J., Reed R., Flynn J., Callihan B., Mattiacci M., Miehle C., Foti G., Lash J.W., Weintraub H., Skeletal myogenesis: The preferred pathway of chick embryo epiblast cells *in vitro*. Dev. Biol., 1996, 173, 279–291.
19. Gerhart J., Baytion M., DeLuca S., Getts R., Lopez C., Niewenhuis R., Nilsen T., Olex S., Weintraub H., George-Weinstein M., DNA dendrimers localize MyoD mRNA in presomitic tissues of the chick embryo. J. Cell Biol., 2000, 149, 825–833.
20. Gerhart J, Neely C, Stewart B, Perlman J, Beckmann D, Wallon M, Knudsen K, George-Weinstein M. Epiblast cells that express MyoD recruit pluripotent cells to the skeletal muscle lineage. J. Cell. Biol., 2004, 164, 739–746.
21. Gros J, Manceau M, Thome V, Marcelle C., A common somitic origin for embryonic muscle progenitors and satellite cells. Nature, 2005, 435, 954–958.
22. Hawke T. J., Garry D.J., Myogenic satellite cells: Physiology to molecular biology. J. Appl. Physiol., 2001, 91, 534– 551.
23. Hughes S.M., Chi M.M.Y., Lowry O.H., Gundersen K., Myogenin induces a shift of enzyme activity from glycolytic to oxidative metabolism in muscles of transgenic mice. J. Cell. Biol., 1999, 145, 633–642.
24. Hughes S.M., Taylor J.M., Tapscott S.J., Gurley C.M., Carter W.J., Peterson C.A., Selective accumulation of myod and myogenin messenger-Rnas in fast and slow adult skeletal – muscle is controlled by innervation and hormones. Development, 1993, 118, 1137–1147.
25. Jackson K.A., Mi T.J., Goodell M.A., Hematopoietic potential of stem cells isolated from murine skeletal muscle. Proceedings of the National Academy of Sciences of the United States of America, 1999, 96, 14482–14486.
26. Jacobs-El J., Zhou M.Y., Russell B., Mrf4, Myf-5, and myogenin messenger-rnas in the adaptive responses of mature rat muscle. Am. J. Phys., 1995, 37, C1045– C1052.
27. Kaehn K., Jacob H.J., Christ B., Hinrichsen K., Poelmann R.E., The onset of myotome formation in the chick. Anat. Embry., 1988, 177, 191–201.
28. Kraus B., Pette D., Quantification of MyoD, myogenin, MRF4 and Id-1 by reverse-transcriptase polymerase chain reaction in rat muscles – Effects of hypothyroidism and chronic low-frequency stimulation. Eur. J. Biochem., 1997, 247, 98–106.
29. Landing B.H., Dixon L.G., Wells T.R., Studies on isolated human skeletal-muscle fibers – including a proposed pattern of nuclear distribution and a concept of nuclear territories. Hum. Pathol., 1974, 5, 441–461.
30. Mauro A., Satellite cell of skeletal muscle fibers. J. Biophys. Biochem. Bytol., 1961, 9, 493–498.

31. McKinney-Freeman S.L., Jackson K.A., Camargo F.D., Ferrari G., Mavilio F., Goodell M.A. Muscle-derived hematopoietic stem cells are hematopoietic in origin. Proceedings of the National Academy of Sciences of the United States of America, 2002, 99,1341–1346.
32. Moss F.P., Leblond C. P., Satellite cells as source of nuclei in muscles of growing rats. Anat. Rec., 1971, 170, 421– 436.
33. Ordahl C.P., Le Douarin N.M., Two myogenic lineages within the developing somite. Development, 1992, 114, 339–353.
34. Ordahl C.P., Berdougo E., Venters S.J., Denetclaw W.F., The dermomyotome dorsomedial lip drives growth and morphogenesis of both the primary myotome and dermomyotome epithelium. Development, 2001, 128, 1731– 1744.
35. Petitte J.N., Karagenc L., Ginsburg M., The origin of the avian germ line and transgenesis in birds. Poult. Sci., 1997, 76, 1084–1092.
36. Pourquie O., Vertebrate somitogenesis. Annu. Rev. Cell Dev. Biol., 2001, 17, 311–350.
37. Relaix F., Rocancourt D., Mansouri A., Buckingham M., A Pax3/Pax7-dependent population of skeletal muscle progenitor cells. Nature, 2005, 435, 948–953.
38. Rosenblatt J.D., Parry D.J., Partridge T.A., Phenotype of adult mouse muscle myoblasts reflects their fiber type of origin. Differentiation, 1996, 60, 39–45.
39. Sadler T., Langmans Medical Embryology. 2000 Lippincott Williams and Wilkins.
40. Schultz E., Satellite cell proliferative compartments in growing skeletal muscles. Dev. Biol., 1996, 175, 84–94.
41. Schultz E., Lipton B.H., Skeletal–muscle satellite cells – changes in proliferation potential as a function of age. Mech. Age Dev., 1982, 20, 377–383.
42. Shefer G., Wleklinski-Lee M., Yablonka-Reuveni Z., Skeletal muscle satellite cells can spontaneously enter, an alternative mesenchymal pathway. J. Cell Sci., 2004, 17, 5393–5404.
43. Smith C.K., Janney M.J., Allen R.E., Temporal expression of myogenic regulatory genes during activation, proliferation, and differentiation of rat skeletal-muscle satellite cells. J. Cell Phys., 1994, 159, 379–385.
44. Spratt N.T., Haas, H., Morphogenetic movements in the lower surface of the unincubated and early chick blastodernm. J. Exp. Zool., 1960, 144, 139–157.
45. Stern C.D., The avian embryo – a powerful model system for studying neural induction. Faseb J., 1994, 8, 687– 691.
46. Stern C.D., Neural induction: old problem, new findings, yet more questions. Development, 2005, 132, 2007–2021.
47. Stern H.M., Hauschka S.D., *In-vitro* somite myogenesis is dependent on neural tube. J. Cell Biochem., 1994, Suppl. 18D, 483–483.
48. Tamaki T., Akatsuka A., Ando K., Nakamura Y., Matsuzawa H., Hotta T., Roy R.R., Edgerton V.R., Identification of myogenic-endothelial progenitor cells in the interstitial spaces of skeletal muscle. J. Cell Biol., 2002a, 157, 571–577.
49. Tamaki T., Akatsuka A., Yoshimura S., Roy R.R., Edgerton V.R. New fiber formation in the interstitial spaces of rat skeletal muscle during postnatal growth. J. Histochem. Cytochem., 2002b, 50, 1097–1111.
50. Voytik S.L., Przyborski M., Badylak S.F., Konieczny S.F., Differential expression of muscle regulatory factor genes in normal and denervated adult-rat hindlimb muscles. Dev. Dyn., 1993, 198, 214–224.
51. Wagers A.J., Conboy I.M., Cellular and molecular signatures of muscle regeneration: Current concepts and controversies in adult myogenesis. Cell, 2005, 122, 659–667.
52. Williams L.W., The somites of the chick. Am. J. Anat., 1910, 11, 55–100.

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