Comparative analysis of myotomal and limb muscles development in selected reptilian representatives (Reptilia).

The comparative analysis of skeletal muscles differentiation in reptiles included studies on trunk muscles development in two snake species: the grass snake (*Natrix natrix*) and the Egyptian cobra (*Naja haje*). Furthermore, limb muscles development of the sand lizard (*Lacerta agilis*) was studied. The light, confocal, and transmission electron microscopy and Western blot method were used.

The analysis in the confocal microscope revealed that during early stages of the grass snake development, the vesicular somites are formed. The cavities of somites (somitocel) are surrounded by the monolayer of Pax3-positive epithelial cells. As somitogenesis proceeds, somites differentiate into the epithelial dermomyotome and mesenchymal sclerotome. In the dermomyotome two lips were distinguished: dorsomedial (DL) and ventrolateral (VL) lips, in epaxial and hypaxial parts of the dermomyotome respectively. Immunocytochemical analysis revealed expression of Pax3 protein in the cells of both lips, whereas Pax7-positive cells were detected in the medial part of the dermomyotome. The primary myotome of the grass snake and the Egyptian cobra is composed of the mononucleated primary myotubes in which myofibrillogenesis has been initiated. The myotome is predominately composed of myotubes, Pax3-positive cells in the vicinity of the ventrolateral lip of the dermomyotome were also observed. The Western blot analysis revealed that the level of Pax3 protein decreased during trunk muscles differentiation in all studied developmental stages (I – VIII). While the Pax7 protein level was comparable in all developmental stages. During the myotomal myogenesis of the grass snake slow (red) and fast (white) muscle fibers occupy similar region of the myotome, as revealed by means of immunodetection of slow and fast isoforms of myosin heavy chains in the myotome.

Studies revealed that in the grass snake and the Egyptian cobra the satellite cells are involved in the muscle growth (hypertrophy and hyperplasia). The satellite cells of the grass snake express Pax7 protein. The presence of subsarcolemmal vesicles in adjacent cells is the indirect, morphological evidence of *N. haje* muscle fibers and satellite cells fusion.

In the advanced stages of myogenesis of the grass snake and the Egyptian cobra two classes of muscle fibers have been distinguished. The class I is composed of typical muscle fibers with myofibrils diffused in the sarcoplasm. Whereas, the class II is characterized by centrally located nucleus surrounded by myofibrils and lipid droplets. Immunocytochemical

analysis revealed the presence slow isoform of myosin heavy chains in the muscle fibers of class II.

During early stages of the sand lizard development, a limb bud is filled with mesenchymal cells surrounded by monolayer epithelium. The progenitor muscle cells migrate from ventrolateral lip of the dermomyotome and form myogenic pools in the limb bud. In the dorsal and ventral parts of myogenic pools Pax3- and Lbx2-positive cells were observed. Immunocytochemical analysis revealed that the cells located in the vicinity of the ventrolateral lip of the dermomyotome were Lbx2-positive. In the contrast to myotomal myogenesis, limb muscles differentiation is asynchronous (premyoblasts, myoblasts, and myotubes were simultaneously observed in myogenic pools). The Western blot analysis revealed the presence of Pax3 and Lbx2-positive cells in all developmental stages of the limb muscle differentiation in the sand lizard (stages 22-32). No Pax7-positive cells were observed in the limb bud, whereas Pax7 protein is present in myotome cells in all analyzed developmental stages.

In conclusion, the structure of somite, the source of progenitor muscle cells, and formation of primary myotome of the grass snake and Egyptian cobra show features common for all amniotes (birds and mammals). The limb muscles development of the sand lizard is similar to the process observed in other Tetrapoda. However, studies revealed unique and characteristic feature of snakes myogenesis. It is assumed, that the lipid droplets-rich class II of red muscle fibers has a different than motoric function. These muscles, capable to lipid storage during myogenesis might be a crucial mechanism for subsequent hibernation in adults. The presence of lipid-rich muscle fibers was observed for the first time during snakes myotomal myogenesis.

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