# HISTOLOGICAL AND MORPHOMETRICAL PARAMETERS OF THE SKELETAL MUSCLE DEVELOPMENT IN PIGS

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Samples of 21, 42-day-old embryos and those from skeletal muscles of newborn and one-day-old piglets were used for the study of the parameters of the skeletal musculature development. The samples were collected from three muscles and processed for the light microscopy. Based upon the preparations subjective conclusions and quantitative evaluation were deduced. It is a case of correlation of the mean thickness of myoblasts, myotubes, mean thickness of muscular fibres and percentual area representation of muscular fibres and connective tissue. Skeletal musculature during prenatal stage develops from the mesoderm. During the early embryonic development promyoblasts arise via mitosis and differentiation of cells of the myotome, which then differentiate to the postmitotic myoblasts. Close approximation of plasma membrane of neighbouring myoblasts initiates fusions and myotubes arise. Development of muscular fibres comes to an end shortly post partum. Origination of new muscular fibres is limited by the number of myosateliocytes that persist among the population of muscular fibres. Subsequently the growth of muscular fibres is carried out by hypertrophy. Fibres and cells of connective tissue are differentiated already in the embryonic stage. Fat droplets in myotubes and interstitial connective tissue are detectable already in the embryonic stage. Septation of skeletal muscles is practically finished post partum.

skeletal muscles; myoblasts; myogenesis; myotubes; muscular fibres; pigs

## INTRODUCTION

In literature there is some information on the development of muscular fibres based upon two theories: unicellular and syncytial. According to the unicellular theory the multinuclear formation is the result of multiple division of the nuclei of one cell (Asai, 1914; Zavarzin, Ščelkunov, 1954). This theory was rejected. According to the syncytial theory the muscular fibre is formed by the fusion of a great number of cells (Holtzer et al., 1958; Aschmore et al., 1973). During the early embryonic development, when the processes of differentiation start, mitoses are the primary mechanism of cellular proliferation. Two types of mitoses can be differentiated. In the early embryonic stage promyoblasts, or presumptive myoblasts arise via mitoses and differentiation of cells of the myotome. The second type of mitoses is immediately linked with the first type and ends with the formation of postmitotic myoblasts. Myoblasts are mononuclear, bipolar, spindle-shaped cells with inclination to the trihedral shape that lost their mitotic ability, but they have ability to synthesise precursors of actin and myosin, and their important feature is ability to connect and form longitudinal formations. Myotubes arise by fusion of myoblasts. In literature they are described as multinuclear syncytium. After fusion in the cytoplasm an intensive synthesis of contractile elements starts with their arrangement into myofibrils on the periphery of myotubes. With increasing number of myofibrils the myotube cytoplasm is filling and migration of nuclei on their periphery occurs. The main sign of the last stage is a shift of nuclei from the centre to the periphery under sarcolemma, while myofibrils fill the interior (Makovický, 2005).

The development of muscular fibres is well-studied as well as described in literature. The individual works present time periods, when myoblasts are connected to myotubes (Lefaucheur et al., 1995). As well, it is described when the ligamentous and fibrous components are differentiated (Gerrard et al., 1999), and conditions under which both septation and vascularisation of muscles are carried out (Dedkov et al., 2001; Picard et al., 2002). The time period of the muscle development in individual species of animals, or human is compared. The time when muscular fibres are differentiated to individual types, including physiology of this process was described, too (Kimball, 2002). Later, in addition to the subjective description the effort for result quantification can be noted. In this sense a lot of different data were described. These come from material, methodology, procedures, technical equipment of laboratories, and lastly also from the way of result interpretation. Objective results could be achieved using the morphometrical methods. In our work the development of skeletal musculature in pigs is described. Subjective conclusions are quantified. During the selected

time period, the values of myoblasts, myotubes, interstitium and fats are measured. Differences in the measurements reflect subjective conclusions. Work of this character has the output with conclusions as for theory as for practice.

## MATERIAL AND METHODS

### Experimental animals

Embryos (at the age of days 21, 42 of development in the prenatal period) and piglets (newborn and 1-day-old) of Pietren breed were selected for the experiment. The basic data are given in Table 1. 21-day-old embryos were obtained from one Pietren breeding sow. 42-day-old embryos were obtained from three Pietren sows. When taking the samples, we followed the Statute of the Slovak government which requires that animal protection be observed during experiments or for other scientific use (Statute 289/2003 § 11, clause 1 and 3).

### Experimental design

In slaughtered sows the cutting in *linea alba* was carried out. The uterus was ligated at the site of cervix, and then the whole extracted with ovaries and oviducts. Then, the uterine horns were cut and all the embryos were extracted. The embryos were marked, packed up in aluminium foil and frozen in liquid nitrogen. In the histological laboratory the scapula (*m. infraspinatus*), part of the back on the level of hips (*m. longissimus dorsi et lumborum*) and thights (*m. rectus femoris*) were extirpated from embryos. Newborn Pietren boars were obtained from the large litter. Practically immediately *post partum* they were euthanatized. One-day-old Pietren boars were similarly obtained from a large litter. After 24 hours *post partum* they were euthanatized and samples from MTB (*m. triceps brachii*), MLD (*m. longissimus dorsi et lumborum*), and MRF (*m. rectus femoris*) were collected, packed up in the aluminium foil and fixed in liquid nitrogen.

### Histological, histochemical examination

Serial cuttings thick 10 μm were cut on the cryo-cut microtome at the temperature of –18 to –21 °C. The first series of samples were stained using haematoxylin-eosin, or toluidin blue, the second series for the evidence of neutral lipids by oil reds "0", for the determination of various types of muscular fibres the sections were incubated for the activity of succinatdehydrogenase (SDH) according to the methodological procedure of S t e i n and P a d y k u l a (1962). The thickness of myoblasts, myotubes, muscular fibres and percentage area representation of fibres and interstitial tissue was determined by the light microscopy Nikon Eclipse E600. Ten sites were randomly selected on every preparation so that minimally 500 fibres from a sample should be evaluated.

## RESULTS

### Analysis of the development of skeletal muscles in 21-day-old embryos

In the preparations there is a body of vertebrates with projections and central canal. The body support is a hyaline cartilage containing chondrocytes and chondroblasts, and on the surface there is a perichondrium formed by the cells and fibres of connective tissue. Laterally, there is a bilaterally visible myotome that forms the base of skeletal muscles. It is separated from the cartilage by a thin small layer of reticular fibres with sparsely placed cells of connective tissue and intercellular space. On its periphery there is a layer of two or three rows of cubic to cylindrical cells that are interconnected. From this layer oval to prolonged cells are released, which are divided. Numerous myoblasts, which are arranged solidly, fit on this part. It is a case of the cells of oval to polygonal shape with centrally put nucleus and a layer of adjacent cytoplasm. Between these cells amorphous matter and sporadic spindle-shaped cells can be seen. In more central parts of the myotome there are some indications of fasciculation with formation of individual muscular bundles. These are separated by amorphous matter with insufficiently numerous cells and a fine plexus of reticular fibres (Fig. 1).

Precise quantification of individual cells is not possible. At the measurement of greater, more centrally placed cells the diameter was measured on the boundary from 4 to 7 μm. Cells greater than 10 μm were found only sporadically. Typical myotubes were not observed.

### Analysis of the development of skeletal muscles in 42-day-old embryos

The light-microscopical pictures of the preparations are from the region of scapula, back, and femur. The bone basis is formed by the hyaline cartilage, where are chondrocytes and chondroblasts. Perichondrium is formed by spindle-shaped cells and fibres. The region of the myotome itself is arranged plexiformly also by the fibrous component, where various cellular component of myoidic elements first of the type myoblasts, or myotubes can be seen. Surrounding component is rich in amorphous matter with areas of the promyoblastic element activation with their mitotic activity. Part of the myotome preparations is

Table 1. Basic data

| Parameter | 21 day-old embryos | 42 day-old embryos | New-born | 1-day-old |
|---|---|---|---|---|
| Number | 5 | 23 | 5 | 5 |
| Parameters | unmeasured | unmeasured | 1.55 kg | 1.60 kg |

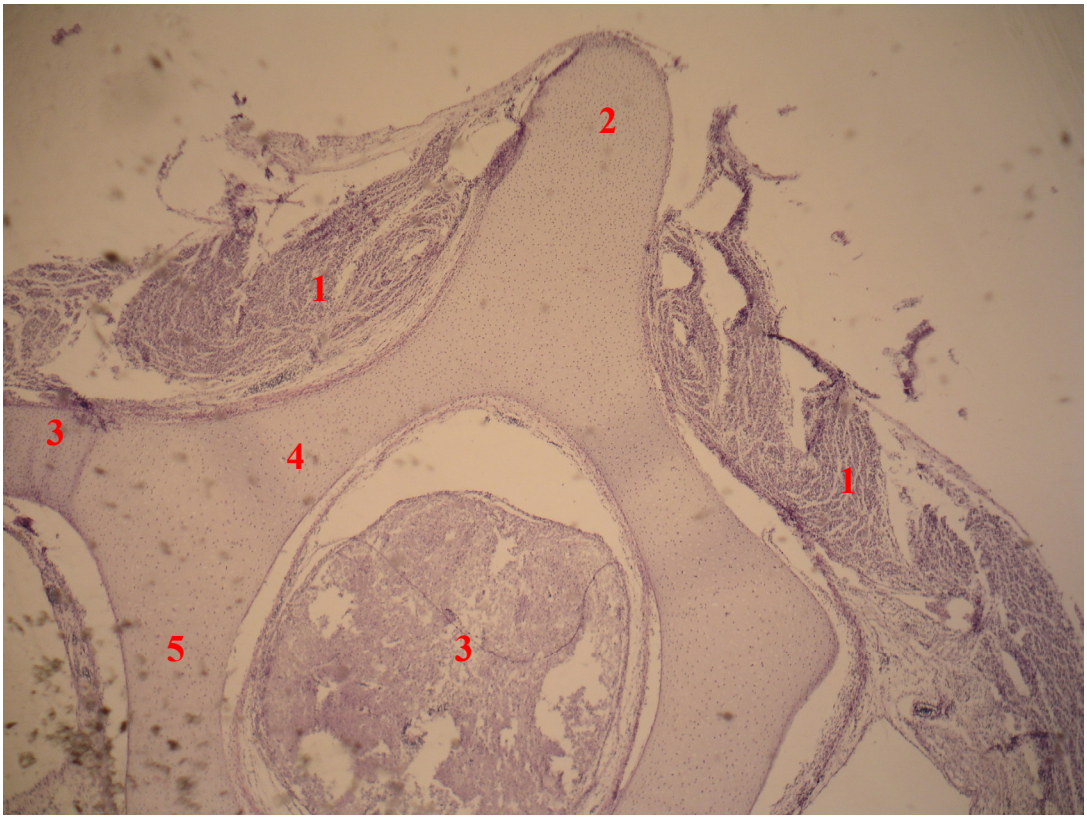


Fig. 1. 21-day-old embryos. Cross sections of body of vertebrates with processus spinosus and central canal. On lateraly side there are visible myotome that forms the base of skeletal muscles MLD.
Legend: HxE, magnification: 40x. 1. The base of dorsal muscles, 2. Procesus spinosus, 3. Foramen vertebralis, 4. Procesus articularis superior, 5. Pediculus arcus vertebrae

Table 2. Mean thickness of myoblasts in 42-day-old embryos (μm)

| Parameter | *x* | Min. | Max. | Variance | *s* | *v* |
|---|---|---|---|---|---|---|
| 1MI-myoblast | 11.60 | 6.15 | 20.26 | 8.64 | 2.94 | 25.34 |
| 2MI-myoblast | 11.65 | 7.22 | 15.45 | 3.93 | 1.98 | 17.01 |
| 3MI-myoblast | 11.30 | 8.48 | 14.47 | 2.61 | 1.62 | 14.30 |
| 1MLD-myoblast | 10.75 | 8.59 | 14.48 | 3.49 | 1.87 | 17.37 |
| 2MLD-myoblast | 11.10 | 8.26 | 16.36 | 3.89 | 1.97 | 17.77 |
| 3MLD-myoblast | 10.75 | 8.48 | 14.65 | 3.49 | 1.87 | 17.37 |
| 1MRF-myoblast | 11.05 | 8.36 | 14.58 | 2.95 | 1.72 | 15.54 |
| 2MRF-myoblast | 11.15 | 8.25 | 14.25 | 3.23 | 1.80 | 16.11 |
| 3MRF-myoblast | 11.65 | 8.78 | 16.15 | 3.03 | 1.74 | 14.94 |
| Total average | 11.22 | 11.23 | 11.49 | 0.11 | 0.34 | 2.99 |

formed by various developmental stages of muscles. Mitoses of promyoblasts, inter-aggregation and fusions of myoblasts, and finally origin of primary and secondary myotubes are detectable. Fusions are initiated by a close contact of plasmatic membranes of two neighbouring myoblasts, when primary myotubes arise. The identical process can be also observed between myotubes under the origin of secondary myotubes. Myoid cells are within the process of septation and formation of primary muscular bundles. Connective tissue component also matures with the presence of reticular fibres with gradual shedding of amorphous matter (Fig. 2). Quantitative data are presented in Tables 2, 3 and 4.

**Analysis of skeletal muscles in newborn and one-day-old piglets**

The histological picture of individual muscles is the same. Subjectively, there are no differences between newborn and one-day-old piglets. Skeletal muscles are demarcated by fibrous sheath. Connective tissue septa protrude from it into the muscle interior that divide the muscle into primary, secondary and tertiary muscular bundles. From the surface of the smallest primary bundles the endomysium penetrates inside in the form of a fine network of reticular fibres that connect with cytoplasm of individual fibres and form around them fine sheaths. Muscular fibres are predominantly of elliptical

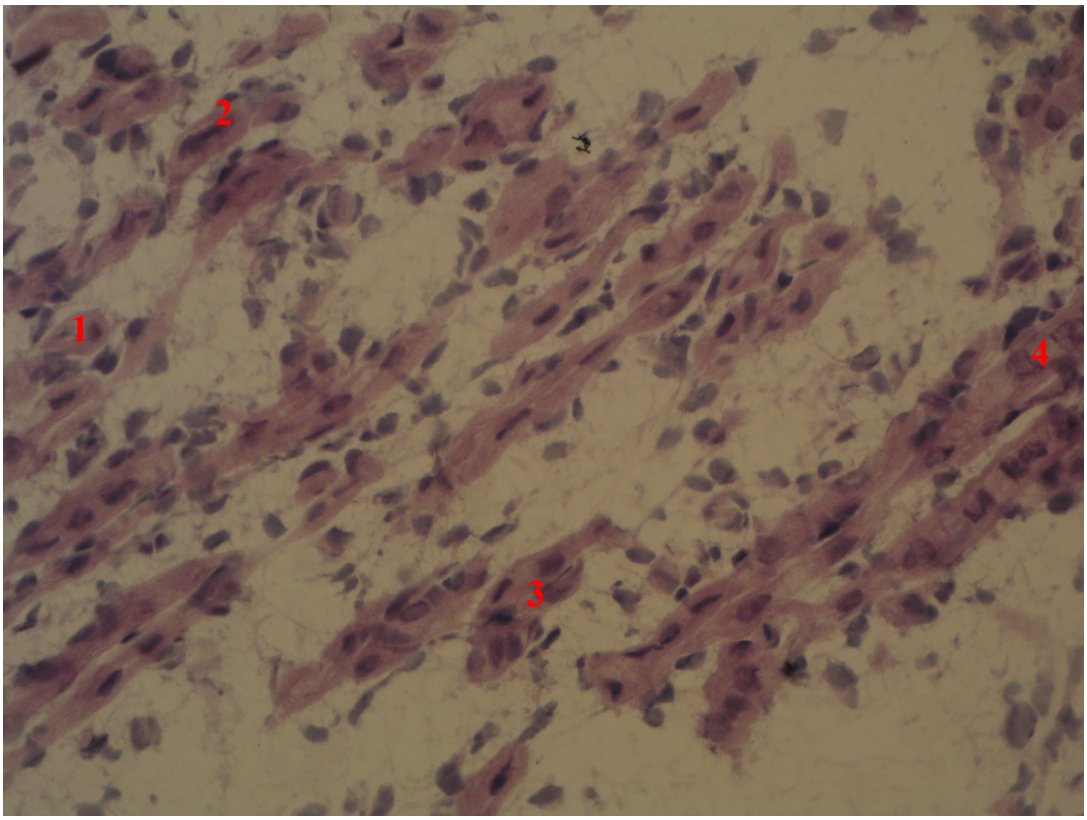


Fig. 2. 42-day-old embryos. A part of myotoms is formed with a various cellular component of myoidic elemets. There are a myoblasts and myotubess can be seen.
Legend: HxE, magnification: 400x 1. Fusions of myoblasts, 2. Primary myotubes, 3. Fusions of secondary myotubes , 4. Secondary myotubes

Table 3. Mean thickness of myotubes in 42-day-old embryos (μm)

| Parameter | *x* | Min. | Max. | Variance | *s* | *v* |
|---|---|---|---|---|---|---|
| 1MI-myotubes | 17.10 | 14.78 | 24.48 | 5.39 | 2.32 | 13.58 |
| 2MI-myotubes | 16.35 | 12.59 | 22.58 | 7.13 | 2.67 | 16.33 |
| 3MI-myotubes | 16.50 | 12.96 | 20.69 | 4.35 | 2.09 | 12.64 |
| 1MLD-myotubes | 17.00 | 14.32 | 22.45 | 3.40 | 1.84 | 10.85 |
| 2MLD-myotubes | 15.65 | 12.15 | 20.78 | 3.73 | 1.93 | 12.34 |
| 3MLD-myotubes | 15.60 | 12.47 | 18.98 | 3.04 | 1.74 | 11.18 |
| 1MRF-myotubes | 16.20 | 12.78 | 18.98 | 3.16 | 1.78 | 10.97 |
| 2MRF-myotubes | 16.10 | 8.95 | 22.65 | 10.19 | 3.19 | 19.83 |
| 3MRF-myotubes | 16.10 | 12.36 | 20.35 | 4.19 | 2.05 | 12.71 |
| Total average | 16.29 | 15.60 | 17.11 | 0.24 | 0.49 | 3.02 |

Table 4. Mean thickness of myoblasts and myotubes in individual muscles of 42-day-old embryos (μm)

| Parameter | *x* | Min. | Max. | Variance | *s* | *v* |
|---|---|---|---|---|---|---|
| MI-myoblast | 11.52 | 11.30 | 11.65 | 0.02 | 0.15 | 1.34 |
| MLD-myoblast | 10.87 | 10.75 | 11.10 | 0.03 | 0.16 | 1.52 |
| MRF-myoblast | 11.28 | 11.05 | 11.65 | 0.07 | 0.26 | 2.33 |
| MI-myotubes | 16.65 | 16.35 | 17.10 | 4.07 | 2.02 | 12.12 |
| MLD-myotubes | 16.08 | 15.60 | 17.00 | 0.42 | 0.65 | 4.03 |
| MRF-myotubes | 16.13 | 16.10 | 16.20 | 0.00 | 0.05 | 0.29 |

to oval shape with greater nuclei on the sarcolemma periphery. On the periphery of primary bundles there are numerous myotubes with nuclei localised centrally. In some places in their sarcoplasma there are some lipid droplets. In the reaction to SDH only red muscular fibres were detected. These were on the basis of subjective judgement classified to β-Red and α-Red (Figs 3 and 4). Quantitative data are presented in Tables 5, 6, 7, 8 and 9.

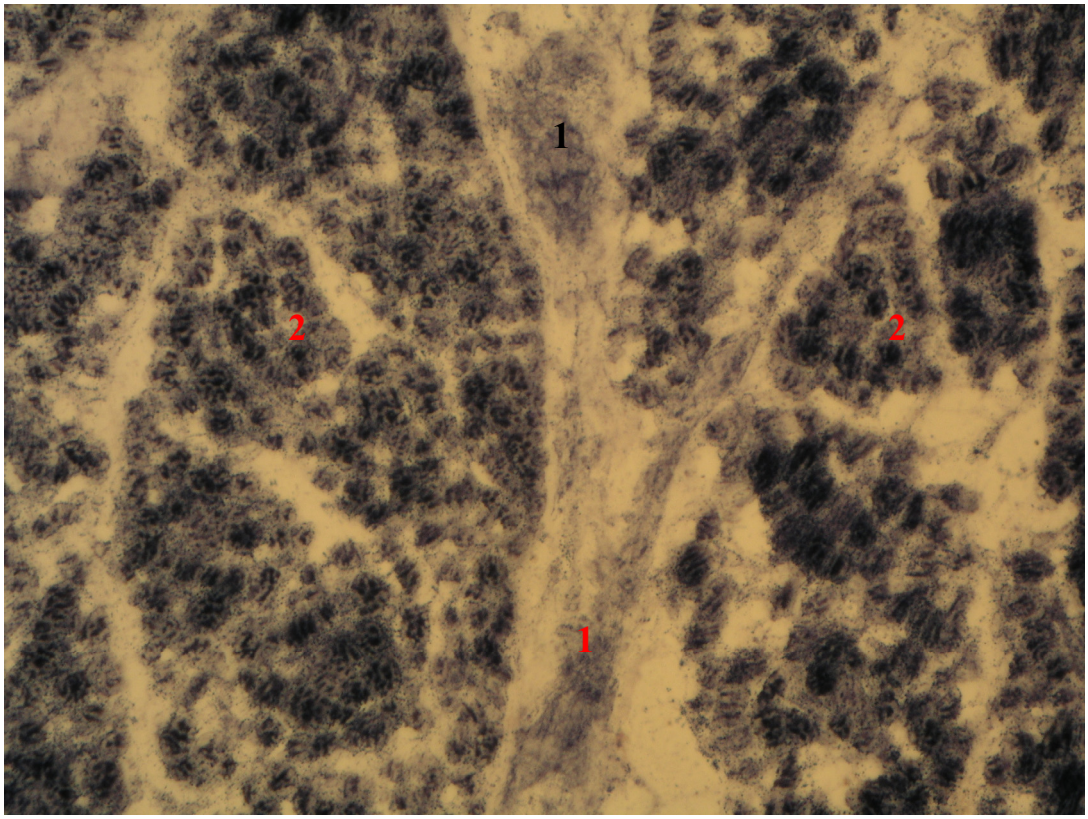


Fig. 3. Newborn pigs. In the reaction SDH only red muscular fibres were detected with a BetaRed a AlfaRed muscular fibres.
Legend: SDH, magnification: 400x 1. Interstitial tissue, 2. Muscular fibres

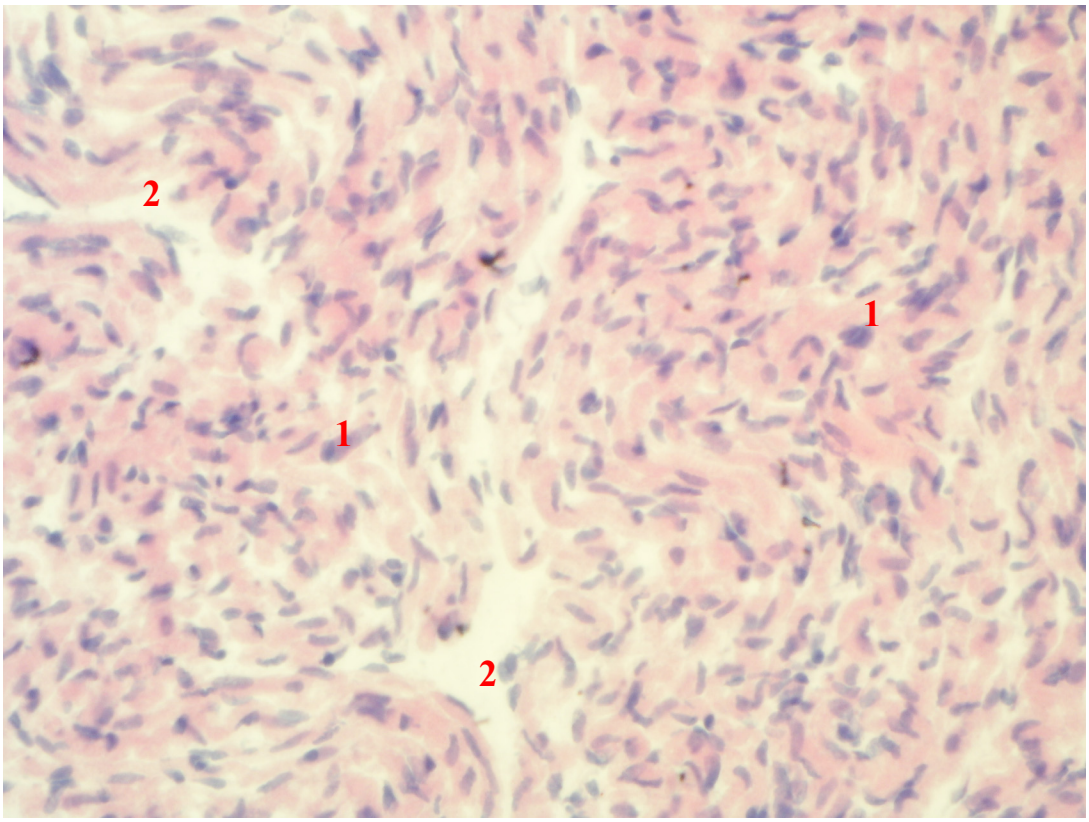


Fig. 4. Muscles of one-day-old pigs. Light-microscopic picture with a cross section of one-day old muscle of pigs.
Legend: HxE, magnification: 400x. 1. Muscular fibres 2. Interstitial tissue

Table 5. Mean thickness of muscular fibres in new-born and one-day-old pigs (μm)

| Parameter | MTB | MLD | MRF | Total average |
|---|---|---|---|---|
| New-born | 17.90 | 20.20 | 20.40 | 19.50 |
| One-day-old | 19.95 | 16.90 | 19.75 | 18.76 |

Table 6. Mean thickness of individual muscular fibres of individual muscles in new-born pigs (μm)

| Parameter | *x* | Min. | Max. | Variance | *s* | *v* |
|---|---|---|---|---|---|---|
| B-Red MTB | 15.90 | 10.00 | 20.00 | 4.59 | 2.14 | 13.47 |
| A-Red MTB | 19.90 | 10.00 | 24.00 | 4.99 | 3.87 | 19.60 |
| B-Red MLD | 18.20 | 14.00 | 26.00 | 8.76 | 2.96 | 16.26 |
| A-Red MLD | 22.20 | 16.00 | 26.00 | 9.56 | 3.09 | 13.93 |
| B-Red MRF | 19.30 | 14.00 | 22.00 | 4.91 | 2.22 | 11.48 |
| A-Red MRF | 21.50 | 16.00 | 26.00 | 10.75 | 3.28 | 15.25 |

Table 7. Mean thickness of individual muscular fibres of individual muscles in one-day-old pigs (μm)

| Parameter | *x* | Min. | Max. | Variance | *s* | *v* |
|---|---|---|---|---|---|---|
| B-Red MTB | 18.70 | 16.00 | 24.35 | 4.91 | 2.22 | 11.85 |
| A-Red MTB | 21.20 | 16.15 | 24.65 | 6.56 | 2.56 | 12.08 |
| B-Red MLD | 18.10 | 14.36 | 22.95 | 4.99 | 2.23 | 12.34 |
| A-Red MLD | 21.10 | 18.68 | 26.48 | 6.19 | 2.49 | 11.79 |
| B-Red MRF | 18.70 | 14.69 | 22.28 | 6.51 | 2.55 | 13.64 |
| A-Red MRF | 20.80 | 16.96 | 26.78 | 12.16 | 3.49 | 16.76 |

Table 8. Percentage area representation of muscular fibres and connective tissue in new-born pigs (%)

| Parameter | IV | Fibres |
|---|---|---|
| MTB | 14.40 | 85.60 |
| MLD | 12.80 | 87.20 |
| MRF | 13.40 | 86.60 |
| Total average | 13.50 | 86.50 |

Table 9. Percentage area representation of muscular fibres and connective tissue in one-day-old pigs (%)

| Parameter | IV | Fibres |
|---|---|---|
| MTB | 12.40 | 87.60 |
| MLD | 12.60 | 87.40 |
| MRF | 13.20 | 86.80 |
| Total average | 12.70 | 87.30 |

## DISCUSSION

In the coloured preparations of 21-old-days embryos somites can be clearly seen, which according to the literature are also termed as myotomes (Wigmore et al., 1996; Chang et al., 1995; Ward, Stickland, 1991). In its most primitive form it has a character of one-layer cubic up to cylindrical epithelium. The cells from myotome are released when their differentiation to the presumptive myoblasts, or also promyoblasts is in progress (Blanton et al., 1999). In compliance with our observations in the light-microscopic picture promyoblasts, myoblasts and also connective tissue cells can be detected in the early embryonic period. Our findings are in agreement with the others (Swatland, 1983; Suelves et al., 2002). Myotubes were not recorded in the preparations. In this period, formation of the bases of individual bundles and perymisium externum was detected. Beermann et al. (1978) observed synchronous formation of primary and secondary muscular bundles. Dedkov et al. (2001) reported that at first the bases of secondary and tertiary muscular bundles are formed, and only in the end the bases of primary bundles. These data are in variance with our observations, when in the preparations it can be seen that from one to two fascicles of aggregated myoblasts at first the bases of primary muscular bundles are formed. The myotomes, however, cannot be observed as reported by other authors (Picard et al., 2002). It can be stated that in addition to the prevailing myoblasts alone, in preparations also cells of other shape are detectable. This fact can document that fibroblasts of the interstitial connective tissue are differentiated already in this period. Regarding this fact it is in agreement with the statement of Gerrard et al. (1999) who reported that differentiation of the interstitial connective tissue runs in correlation with differentiation of skeletal musculature.

In the preparations of 42-day-old embryos numerous mitoses of promyoblasts, myoblast fusions, primary, and partially secondary myotubes can be observed. In the preparations from the femoral muscles (MRF) also numerous lipid droplets in the sarcoplasma of myotubes as well as in the interstitial ligament can be seen. In the other parts the lipid droplets were detectable only in the myotubes sarcomplasma. Swatland (1983) described in detail the process of myoblast fusion, and he distinguished several stages. According to his observation, in the first stage myoblasts closely come near to each other by cytoplasmatic membranes, and by their inter-touching they fuse and cy-

toplasm is mixed. The myoblast cytoskelet is mixed and myotubes arise. The myotube nuclei stay in the centre of sarcoplasm and they are surrounded by myofibrils. Remaining promyoblast mitoses at the fusions of myoblasts was also confirmed by other authors (Lobo et al., 1999; Lefaucheur et al., 1995). Duxson and Sheard (1995) reported that the number of muscular elements in the primary bundles increases up to parturition. Rehfeldt et al. (2001) at the study of differentiation of skeletal musculature recorded occurrence of myotubes in 31-day-old pig embryos. Ashmore et al. (1973) detected primary myotubes in 35-day-old pig embryos, and secondary in 60-day-old ones. Beermann et al. (1978) observed the presence of secondary myotubes already in 45-day-old embryos in secondary bundles of the developing muscular fibres. According to Paul et al. (2004), however, the first myotubes occur after close aggregation of myoblasts into inter-parallel rows. It can be observed that the bases of primary muscular bundles are already originated and they form the bases of secondary muscular bundles. Dedkov et al. (2001) term those as secondary, which only arose impression of primary muscular bundles. Picard et al. (2002), however, reported that the number of differentiating muscular fibres in the primary bundles will be comprised by differentiation of secondary and tertiary myotubes.

Harlizius and Shellander (2002) presented the results of the histochemical study of the research of prenatal muscles, and they identified following developmental points: beginning of myogenesis on day 14, starting of terminal interferentiation on day 21, origin of primary myotubes on day 35, formation of slow (β-Red) on day 49, origin of secondary myotubes on day 63, distinguishing of two types of fibrils according to the myosine ATP activity on day 77, and muscular hypertrophy on day 91. Ashmore et al. (1973) detected the activity of myofibrillar ATP on the periphery of myotubes already on day 50 of prenatal development of pigs. In our work we describe the occurrence of secondary myotubes in 42-day-old embryos, while the SDH reaction in this period was lightly positive. McPherson et al. (2004) carried out the analysis from 320 embryos at the age categories: 45, 60, 75, 90, 102, and 110 days and were collected from 33 pregnant sows. Based upon their statement it can be stated that in the preparations of 45-day-old prenatal embryos the secondary myotubes were already well detectable.

The process of differentiation of myotubes from myoblasts is accompanied by formation of secondary and tertiary muscular bundles, and connective tissue plays a significant role at differentiation of striated skeletal muscles. By our observations, in the early embryonic period connective tissue component is formed only by cells and colourless amorphous matter. Subsequent formation of interstitial tissue is gradual, when at the beginning reticular fibres prevail, and then collagenous fibres dominate. The percentage proportion of interstitial tissue itself, however, in early embryonic period does not exceed that of individual muscular cells, while its portion due to the growth of pig embryos decreases. This fact is also documented by the results of another author (Gerrard et al., 1999).

Adipose connective tissue is also a part of striated skeletal muscles. Adipocytes with abundant content of lipids are its basic unit. As according to Burrin et al. (1997) skeletal muscles of new-born piglets are characterised by the capability of growth and analogically also with the potential of high utilisation of nutrients, it is apparent that due to the growth of skeletal muscles in this period the fat depots were not deposited in them. On day 1 post partum the muscular fibres are characterised by a great content of lipid droplets that are distributed in the sarcoplasm. They appear in the connective tissue only rarely, between tertiary bundles, but with age their number increases, especially in surroundings of vessels. To the smallest extent they are localised between primary, and to the largest extent between tertiary muscular bundles (Mellor, Cockburn, 1986; Gerfault et al., 2000). Lipid cells have not been described in new-born and one-day-old boars, what corresponds with the results of other authors (Davis et al., 2000; Sillence et al., 2002). With age concentration of intramuscular fat increases in the red fibres that can be in harmony with the results of da Costa et al. (2004). Gerfault et al. (2000) reported that subcutaneous and fat tissues in the muscles of pigs are well detectable already in 7-day-old pigs. In the studies regarding this problem, above all, the presence of individual substances that manage to stimulate synthesis of proteins and lipids in the neonatal skeletal muscles is pointed out (Davis et al., 2002; Suryawan, Davis, 2003).

Myogenesis of individual muscles in pigs is post partum basically finished, while the histological picture in this period documents the classical structure of skeletal muscles. This statement is deduced from the presence of sporadic myotubes, thus muscular fibres with centrally localised nuclei. These observations are in harmony with those of other authors (Zoecklein et al., 1994; Kimball, 2002). Swatland (1983) reported that animals are born with a certain number of muscular fibres that grow to length and thickness. Based upon our results it can be stated that sporadic myotubes with pronounced localisation of nuclei close to sarcolemma are also detectable in new-born boars.

Uhrín et al. (1986) studied the skeletal musculature in pigs in order to reveal the status of differentiation and distinguishing of individual types of muscular fibres. They reported that individual types of muscular fibres are not histochemically distinguishable at birth and shortly after it, which corresponds with our results. Kaman (1995a) investigated seven topographically and functionally various muscles in ten in every group of new-born wild, primitive and domesticated swine. According to his observation the muscular fibre was fully morphologically and histochemically differentiated in neonates of wild swine and primitive swine, but in the group of domesticated pigs the myofibril deficiency of sporadic muscular fibres was observed. In other work Kaman (1995b) described on the basis of histochemical detection of the ATP-ase activity the SDH reactions and glycogen determination of type distribution of muscular fibres in new-born pigs. Based upon his results it can be stated that muscular fibres of

new-born pigs were morphologically and histochemically adequately differentiated. Based upon ATP-ase all three types of fibres were distinguishable, but SDH reaction did not make objective type identification of individual muscular fibres possible in piglet to the age of one-month. As well, P e s t o v et al. (2001) reported that individual muscular fibres in new-born piglets on the basis of ATP-ase are distinguishable only after the birth. Similarly A l a - b a y et al. (1995, 1996) recommend only application of the ATP-ase method for objective distinguishing of the individual muscular fibres.

Even if it does not unambiguously follow from the conclusions, the existence of stem cells capable of changing to muscular fibres is expected. This theory could be confirmed only by identification of the structure of integral proteins of plasmatic membrane of muscular fibres. Then, the conformation of similar proteins in cells could be looked for. Origin of new muscular fibres already during life would open new possibilities in clinical practice. It is a case of therapeutic possibilities at aplasia of skeletal muscles, at muscular dystrophies up to bio-application potentials in animal production.

## CONCLUSIONS

Histological and morphometric parameters of the development of skeletal muscles in pigs are described. From the results it follows that in 21-day-old embryos the myoblastic stage can be detected. Bases of *perimysium externum* are formed. Fibres and cells of connective tissue are differentiated already in this period. In the myotome of skeletal muscle in 42-day-old embryos promyoblasrs, myoblasts, primary and secondary myotubes can be observed. Bases of primary and secondary muscular bundles are formed. As well, lipid droplets are visible in the myotube sarcoplasm and in the interstitial tissue. In the skeletal muscles of new-born and one-day-old piglets apparently differentiated muscular fibres as well as myotubes can be seen. Septation of muscular fibres to bundles is finished. The SDH reaction is positive for red muscular fibres. Myogenesis goes on also in the *post partum* period, while the origin of new muscular fibres is limited by the number of myosateliocytes that persist among the muscular fibre population.

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## REFERENCES

ALABAY, B. – SAGLAM, M. – YORUK, M. – ERGUN, L.: Studies on the adenosinetriphosphatase activity in the extrafusal and intrafusal fibres of the anterior and posterior latissimus dorsi muscles of quails. Vet. Fakult. Derg., *42*, 1995: 295–300.

ALABAY, B. – SAGLAM, M. – OZCAN, Z. – ALTUNAY, H. – ERGUN, L.: Comparative study in the extra and intrafusal muscle fibres of the ilofibular and biventer muscles in quails. Vet. Fakult. Derg., *43*, 1996: 139–145.

ASAI, T.: Beitrage zur Histologie und Histogenese der quegesttreitten Musculatur der Saugentiere. Arch. Mikr. Anatom., *8*, 1914: 86–87.

ASHMORE, C. R. – ADDIS, P. S. – DOER, L.: Development of muscle fibers in the fetal pig. J. Anim. Sci., *36*, 1973: 1088–1093.

BEERMANN, D. H. – CASSENS, R. G. – HAUSMAN, G. J.: A second look at fiber type differentiation in porcine skeletal muscle. J. Anim. Sci., *46*, 1978: 125–132.

BLANTON, J. R. – GRANT, A. L. – McFARLAND, D. C. – ROBINSON, J. P. – BIDWELL, C. A.: Isolation of two populations of myoblasts from porcine skeletal muscle. Muscle Nerve., *22*, 1999: 43–50.

BURRIN, D. G. – DAVIS, T. A. – FIOROTTO, M. L. – REEDS, P. J.: Role of milk-borne vs endogenous insulin-like growth factor I in neonatal growth. J. Anim. Sci., *75*, 1997: 2739–2743.

CHANG, K. C. – FERNANDES, K. – CHANTLER, P. D.: Cloning and in vivo expression of the pig MyoD gene. J. Muscle Res. Cell Motil., *16*, 1995: 243–247.

daCOSTA, N. – McGILLIVRAY, C. – BAI, Q. – WOOD, J. D. – EVANS, G. – CHANG, K. C.: Restriction of dietary energy and protein induces molecular changes in young porcine skeletal muscles. J. Nutr., *134*, 2004: 2191–2199.

DAVIS, T. A. – NGUYEN, H. V. – SURYAWAN, A. – BUSH, J. A. – JEFFERSON, L. S. – KIMBALL, S. R.: Developmental changes in the feeding-induced stimulation of translation initiation in muscle of neonatal pigs. Am. J. Physiol. Endocrinol. Metab., *279*, 2000: E1226–1234.

DAVIS, T. A. – FIOROTTO, M. L. – BURRIN, D. G. – VANN, R. C. – REEDS, P. J. – NGUYEN, H. V. – BECKETT, P. R. – BUSH, J. A.: Acute IGF-I infusion stimulates protein synthesis in skeletal muscle and other tissues of neonatal pigs. Am. J. Physiol. Endocrinol. Metab., *283*, 2002: E638–647.

DEDKOV, E. I. – KOSTROMINOVA, T. Y. – BORISOV, A. B. – CARLSON, B. M.: Reparative myogenesis in long-term denervated skeletal muscles of adult rasts results in a reduction of the satellite cell population. Anat. Rec., *263*, 2001: 139–154.

DUXSON, M. J. – SHEARD, P. W.: Formation of new myotubes occurs exclusively at the multiple innervation zones of an embryonic large muscle. Dev. Dyn., *204*, 1995: 391–405.

GERFAULT, V. – LOUVEAU, I. – MOUROT, J. – Le DIVIDICH, J.: Lipogenic enzyme activities in subcutaneous adipose tissue and skeletal muscle from neonatal pigs consuming maternal or formula milk. Reprod. Nutr. Dev., *40*, 2000: 103–112.

GERRARD, D. E. – OKAMURA, C. S. – GRANT, A. L.: Expression and location of IGF binding proteins-2, -4, and -5 in developing fetal tissues. J. Anim. Sci., *77*, 1999: 1431–1441.

HARLIZIUS, B. K. – SHELLANDER: Identification of genes controlling pork quality. In: 28th Conf. ISAG, 11–15 August 2002. Göttingen, Germany.

HOLTZER, H. – ABOTT, J. – LASH, J.: On formation of multinucleated myotubec. Anat. Record., *131*, 1958: 867.

KAMAN, J.: Morphological and histochemical type differentiation of muscle-fibers in new-born pigs – its relationship to topical myopaties. Acta Vet. Brno, *64*, 1995a: 35–38.

KAMAN, J.: Postnatal morphological and histochemical type differentiation of the porcine muscle fibres. Acta Vet. Brno, *64*, 1995b: 211–218.

KIMBALL, S. R.: Regulation of global and specific mRNA translation by amino acids. J. Nutr., *132*, 2002: 883–886.

LEFAUCHEUR, L. – EDOM, F. – ECOLAN, P. – BUTLER-BROWNE, G. S.: Pattern of muscle fiber type formation in the pig. Dev. Dyn., *203*, 1995: 27–41.

LOBO, M. V. – SANTANDER, R. G. – CUADRADO, G. M., ALONSO, F. J.: Cytochemical localization of calcium in prefusion myoblasts from the chick embryo myotome. Histochem. J., *31*, 1999: 347–355.
MAKOVICKÝ, P.: The development and histogenesis of cross striated skeletal muscles of pigs. [PhD. thesis.] SPU, Nitra, Slovak Republic, 2005. (In Slovak)
McPHERSON, R. L. – JI, F. – WU, G. – BLANTON, J. R. – KIM, S. W.: Growth and compositional changes of fetal tissues in pigs. J. Anim. Sci., *82*, 2004: 2534–2540.
MELLOR, D. J. – COCKBURN, F.: A comparison of energy metabolism in the new-born infant, piglet and lamb. Q. J. Exp. Physiol., *71*, 1986: 361–379.
PICARD, B. – LEFAUCHEUR, L. – BERRI, C. – DUCLOS, M. J.: Muscle fibre ontogenesis in farm animal species. Reprod. Nutr. Dev., *42*, 2002: 415–431.
PAUL, A. C. – SHEARD, P. W. – DUXSON, M. J.: Development of a mammalian series-fibered muscle. Anat. Rec. A Discov. Mol. Cell. Evol. Biol., *278*, 2004: 571–578.
PESTOV, N. B. – KORNEENKO, T. V. – ZHAO, H. – ADAMS, G. – KOSTINA, M. B. – SHAKHPARONOV, M. I. – MODYANOV, N. N.: The betam protein, a member of the X, K-ATPase beta-subunits family, is located intracellularly in pig skeletal muscle. Arch. Biochem. Biophys., *396*, 2001: 80–88.
PICARD, B. – LEFAUCHEUR, L. – BERRI, C. – DUCLOS, M. J.: Muscle fibre ontogenesis in farm animal species. Reprod. Nutr. Dev., *42*, 2002: 415–431.
REHFELDT, C. – KUHN, G. – VANSELOW, J. – FURBASS, R. – FIEDLER, I. – NURNBERG, G. – CLELLAND, A. K. – STICKLAND, N. C. – ENDER, K.: Maternal treatment with somatotropin during early gestation affects basic events of myogenesis in pigs. Cell Tissue Res., *306*, 2001: 429–440.
SILLENCE, M. N. – MUNN, K. J. – CAMPBELL, R. G.: Manipulation of growth in pigs trough treatment of the neonate with clenbuterol and somatotropin. J. Anim. Sci., *80*, 2002: 1852–1862.
STEIN, J. M. – PADYKULA, H. A.: Histochemical classification of individual skeletal muscle fibres of the rat. Am. J. Anat., *110*, 1962: 103–123.
SUELVES, M. – LÓPEZ-ALEMANY, R. – LIUÍS, F. – ANIORTE, G. – SERRANO, E. – PARRA, M. – CARMELIET, P. – MUÑOZ-CÁNOVES, P.: Plasmin activity is required for myogenesis in vitro and skeletal muscle regeneration in vivo. Blood, *99*, 2002: 2835–2844.
SURYAWAN, A. – DAVIS, T. A.: Protein-tyrosine-phosphatase 1B activation is regulated developmentally in muscle of neonatal pigs. Am. J. Physiol. Endocrinol. Metab., *284*, 2003: E47–54.
SWATLAND, H. J.: The histochemistry of very small muscle fibres in growing skeletal muscles. J. Neurol. Sci., *61*, 1983: 435–442.
UHRÍN, V. – KULÍŠKOVÁ, Ľ. – POLTÁRSKY, J. – BULLA, J.: Microscopic structure of some muscles in pigs included in the hybridization program. Živoč. Výr., *31*, 1986: 839–847.
WARD, S. S. – STICKLAND, N. C.: Why are slow and fast muscles differentially affected during prenatal undernutrition. Muscle Nerve, *14*, 1991: 259–267.
WIGMORE, P. M. – MALEKI, F. – EVANS, D. J. – McERLAIN, M.: After embryonic day 17, distribution of cells on surface of primary muscle fibres in mouse is non-random. Dev. Dyn., *207*, 1996: 215–221.
ZAVARZIN, A. A. – ŠČELKUNOV, I. S.: Rukovodstvo po gistologii. Leningrad, 1954.
ZOECKLEIN, L. J. – LAKE, D. S. – LOPEZ, J. R. – GALLANT, E. M. – TAYLOR, S. R.: Ca2+-relase and contraction in myotubes from normal and malignant hyperthermia susceptible (MHS) neonatal pigs. J. Gen. Phys., *104*, 1994: A23–A24.

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**Histologické a morfometrické ukazovatele vývinu kostrových svalov ošípaných.**

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Pre štúdium ukazovateľov vývinu kostrovej svaloviny sme v práci použili vzorky z 21,42dňových embryí a vzorky z kostrových svalov novorodených a jednodňových prasiatok. Vzorky boli odobraté z troch svalov a spracované pre potreby svetelnej mikroskopie. Z vyhotovených preparátov sú vyvodené subjektívne závery a kvantitatívne hodnotenia. Ide o koreláciu priemernej hrúbky myoblastov, myotúb, priemernú hrúbku svalových vlákien a percentuálne plošné zastúpenie svalových vláken a väziva. Kostrová svalovina sa počas prenatálneho štádia vyvíja zo stredného zárodočného listu mezodermy. V priebehu raného embryonálneho vývoja vznikajú mitózami a diferenciáciou buniek myotómu promyoblasty, ktoré sa diferencujú na postmitotické myoblasty. Tesným priblížením plazmatickej membrány susediacich myoblastov sa iniciujú fúzie a vznikajú myotuby. Vývoj svalových vláken sa končí krátko post partum. Vznik nových svalových vláken je limitovaný počtom myosateliocytov, ktoré perzistujú medzi populáciou svalových vláken. Následne sa rast svalových vláken uskutočňuje hypertrofiou. Vlákna a bunky väziva sa diferencujú už v embryonálnom období. Tukové kvapôčky v myotubách a interstitiálnom väzive sú detekovateľné už v embryonálnom období. Septácia kostrových svalov je post partum prakticky ukončená.

kostrové svaly; myoblasty; myogenéza; myotuby; svalové vlákna; ošípané

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