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Morphology and histochemistry of muscle fiber types of skeletal muscle tissue of lambs during growth

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ABSTRACT. Morphological and histochemical methods were used to study the degree of development of myofibers of *semitendinosus* muscle of lambs aged from birth to the slaughter period. Based on NADH-TR and m-ATPase, after alkaline and acid preincubations, muscle fibers were classified as SO, FOG and FG types. At birth, slow oxidative (SO) myofibers were clearly distinguished from fast myofibers. FOG and FG myofibers were not clearly visualized. At weaning and slaughter, fast myofibers were clearly subdivided into FOG and FG types. At birth, ultrastructural examination revealed myofibers with different degrees of maturation and a great number of satellite cells. At weaning, the majority of myofibers presented a differentiated aspect. Myofibers with variable degrees of differentiation and satellite cells were still frequent. At slaughter, despite the majority of myofibers revealed a mature and hypertrophic pattern, a great number of immature muscle cells was observed. This observation seems to indicate that the rate of growth is still active.

Key words: lambs, myofibers, histochemistry, morphology.

RESUMO. Morfologia e histoquímica das fibras musculares do tecido muscular esquelético de cordeiros durante o crescimento. Métodos morfológicos e histoquímicos foram utilizados para se estudar o grau de desenvolvimento das fibras musculares do músculo *semitendinosus* de cordeiros, em diferentes idades, do nascimento ao abate. Baseados no NADH-TR e m-ATPase, após pré-incubação alcalina e ácida, as fibras musculares foram classificadas como SO, FOG e FG. Ao nascimento, as fibras *slow* (SO) foram claramente distinguidas das fibras *fast.* As fibras FOG e FG não foram visualizadas claramente. Ao desmame e abate, essas fibras foram facilmente subdivididas nos tipos FOG e FG. Ao nascimento, a análise ultraestrutural revelou, fibras com diferentes estágios de maturação e grande número de células satélites. Ao desmame, a maioria das fibras apresentava aspecto diferenciado. Fibras em vários graus de diferenciação e células satélites ainda eram freqüentes. Ao abate, apesar da maioria das fibras apresentar padrão hipertrófico e maduro, um grande número de fibras imaturas foi observado. Essa observação parece indicar que o crescimento muscular é ainda bastante ativo.

Palavras-chave: cordeiros, fibras musculares, histoquímica, morfologia.

In mammals, skeletal muscles are composed of a mixed population of red, intermediate and white muscle fibers (Close, 1972). Despite the large variability in muscle fiber composition, the meat quality is strongly influenced by the predominance of a specific myofiber type, the size and frequency of occurrence (Ashmore, 1974; Ross and Romrell, 1993; Dwyer *et al.*, 1994).

In addition to morphological methods, fiber differences can be visualized by the use of enzymatic tests, in which the metabolic, contractile and physiological abilities of myofibers are clearly demonstrated (Peter *et al.*, 1972). Based on the morphology and nicotinamide adenine dinucleotíde tetrazolium reductase (NADH-TR) and myofibrilar ATPase (m-ATPase) reactions, myofibers were classified in three main groups, with the following nomenclature and characteristics:

SO (slow twitch oxidative): small diameter, low level of glycolytic enzymes, moderate to high degree

of aerobic ability and strong reaction to nicotinamide adenosine dinucleotíde tetrazolium reductase (NADH-TR) and m-ATPase after acid preincubation (pH 4.7) and negative after alkaline preincubation (pH 9.4).

FOG (fast twitch oxidative glycolytic): intermediate diameter, moderate to high aerobic activity, together with a high concentration of cytochrome and oxidative enzymes. This kind of myofibers reveals an intermediate reaction to NADH-TR and m-ATPase after alkaline (pH 9.4) preincubation and negative reaction after preincubation at pH 4.7.

FG (fast twitch glycolytic): larger diameter, high level of glycogen, low levels of both cytochrome concentration and oxidative activity. These myofibers reveal weak reaction to NADH-TR, strong reactions to m-ATPase after alkaline (pH 9.4) preincubation and intermediate reaction after acid preincubation (pH 4.7).

In this area of investigation, the use of morphological, metabolic and contractile methods, in addition to determining myofibers composition of a muscle, is important in the determination of their degree of maturation. In addition, the knowledge of the degree of differentiation of muscle fibers constitutes one of the most important parameters to evaluate the economic period of slaughter of a species.

The importance of the knowledge of microscopic characteristics in the determination of myofiber types has been stated by several works, specially the parameters concerning meat quality (Lawrie, 1974). According to this author, meat quality is largely influenced by the changes in the morphology and muscle fiber composition during growth.

The adoption of morphological and histochemical methods was also useful in the study of changes in myofiber size, distribution, frequency and connective tissue of different breeds of lambs (White *et al*., 1978; Sivachelvan and Davies, 1986; Suzuki and Tamate, 1988),

Based on the above informations, the present paper deals with the evaluation of morphological, metabolic and physiological characteristics of muscle tissue of lambs along successive stages of growth.

Material and methods

Using surgical methodology, several samples were collected from the superficial region of *semitendinosus* muscle of four lambs, ½ Corriedale + ½ Bergamácia. Sampled muscle tissues were collected at birth, at weaning (two months old), and at the slaughter period, where live body weight reached 30 to 32 kg.

After this, samples were kept at room temperature for 15 min (Khan, 1977). The material was then cut in small segments of about 1 cm x 0,5 cm. and plunged into n-Hexane, precooled to -70ºC with liquid Nitrogen (Chayen *et al.,* 1969). The frozen material was then transferred to a cryostat chamber at -20ºC. In order to equilibrate with the higher temperature, the material was stored in this environment for 1 hr (Pullen, 1977).

Individual blocks were then fixed to the metallic chucks of the cryostat, using a special adhesive, the OCT Tissue TEK. In this phase, the blocks were oriented to obtain transverse sections of muscle fibers.

Several serial sections with 8 µm thickness were obtained. For the evaluation of morphological aspects of the tissue, the first series of sections was stained with hematoxylin and e eosin (HE), according to Lillie, (1954). For the evaluation of oxidative-glycolytic metabolism of myofibers, a subsequent series was submitted to the demonstration of nicotinamide adenine dinucleotide tetrazolium reductase (NADH-TR), according to the methodology of Pearse (1968), modified by Dubowitz and Brooke (1973). In order to test the fast and slow contraction abilities of myofibers, the myofibrilar ATPase (m-ATPase), after both acid (pH 4.3 to 4.6) and alkaline preincubations (pH 9.4 to 10.4), (Padykula and Herman, 1955) was carried out. Myofibers were classified as SO (slow twitch oxidative), FOG (fast twitch glycolytic) and FG (fast twitch glycolytic), according to Peter *et al*. (1972).

For ultrastructural analysis, muscle samples were fixed in 2.5% glutaraldehyde in 0.1M sodium phosphate buffer, pH 7.3 at 4ºC, washed in the same buffer, postfixed in 1% osmium tetroxide, embedded in araldite and then sectioned with 60-80 nm thickness.

Results and discussion

In this study, type SO myofiber revealed a strong reaction to NADH-TR and m-ATPase, after acid preincubation and negative reaction after alkaline preincubation. Type FOG myofiber revealed moderate intensity of reaction to NADH-TR and m-ATPase, after alkaline preincubation and weak to moderate after acid preincubation. Type FG myofiber revealed weak reaction to NADH-TR and strong reaction to m-ATPase, after alkaline preincubation and weak reaction after acid preincubation.

At birth, *semitendinosus* muscle revealed to be composed of large number of myofibers arranged in

distinct fascicles (Figure 1a). The majority of myofibers were round in shape presenting a small diameter and an acidophilic pattern of staining. One or more nuclei were observed in the peripheric cytoplasm region. In some myofibers, the nuclei were centrally located. The m-ATPase reaction demonstrated the contraction ability of fibers. When the reaction was previously incubated in alkaline medium, some fibers revealed negative reaction. This finding is indicative of the presence of slow myosin isoform. This pattern of reaction was more frequent in the fibers with an intermediate size. In the fibers remaining, this reaction changed their intensity from moderate to strong. This pattern of reaction indicates the presence of fast miosin isoform. However, when this reaction was accomplished after acid preincubation, an inverse pattern of reaction could be observed (Figure 1b).

When the m-ATPase reaction was used, the slow contracting fibers were easily distinguished from the fast contracting ones (Figure 1b). However, these latter fibers could not be classified into FOG and FG subtypes. This characteristic was similar to the findings of White *et al*. (1978) and Suzuki and Cassens (1983).

It is well known that, in the immediate postnatal period, significative histochemical changes in myofiber types are observed in most mammalian muscles (Davies, 1972). In this field of investigation, in new born dogs, m-ATPase made the visualization of FOG and FG fibers as distinct types (Braund *et al*., 1978) possible. Similar findings were described in bovines (Suzuki, 1976) and in pigs (Suzuki and Cassens, 1980). However, similar observations could not be seen in sheep (Suzuki, 1976).

According to Suzuki and Cassens (1983), in sheep muscles, the differentiation of FOG and FG myofibers is accomplished between birth and two-week period. According to these authors, the visualization of distinct myofiber types occurs from 8 to 10 postnatal weeks. Moody *et al*. (1980) attributed this pattern of reaction to the fact that myosin composition is not yet fixed at birth.

According to the present results, at birth, in the sections reacted for the demonstration of NADH-TR, which reveals oxidative-glycolytic metabolism, this ability shows to be variable, where both the pattern of reaction and the distribution of the end product of the reaction, the formazan, were not clearly evident (Figure 1c). This observation is indicative of some degree of muscle fiber immaturity. Despite the reactivity being more intense in some myofibers (slow), it was not possible to distinguish a clear cut division between FOG and FG fibers. These observations were coincident with that of Suzuki and Cassens (1983). According to the literature, muscle fibers with characteristics similar to the above descriptions were classified as undifferentiated (Dubowitz and Brooke, 1973).

At birth, the ultrastructural analysis revealed a mixed population of myofibers with different degrees of maturation. Intermediate and large fibers, presenting the cytoplasm filled with myofibrils, and the nucleus located in the subsarcolemmal region were classified as mature myofibers. A relatively large number of small and undifferentiated fibers were observed in the connective tissue (Figure 3a). These fibers revealed a central nucleus, scarce myofibrils dispersed in the central area of the cytoplasm and a large number of polyribosomes. At this age, associated with the mature muscle fibers, a large number of satellite cells (Figure 3b), located between basal lamina and the plasma membrane, was observed. At ultrastructural level, the characteristics of muscle fibers of lambs here described are similar to those of most mammals (Stromer *et al*., 1974).

At weaning, the majority of muscle fibers revealed a hypertrophic aspect, presenting a. preferentially polygonal outline. The frequency of small and undifferentiated myofibers was still relatively high. The reactivity to m-ATPase, after acid and alkaline preincubations showed distinct SO, FOG and FG fibers, presenting a typical reverse pattern of reactivity. At the same time, the pattern of NADH-TR reaction was characteristic for each kind of myofiber. The reaction was particularly intense in the subsarcolemmal cytoplasm of type SO myofibers. The ultrastructural analysis revealed an increased number of fibers with mature aspects. The frequency of small (Figure 3c) and undifferentiated myofibers was less significant.

At the slaughter, the majority of muscle fibers revealed mature and hypertrophic characteristics, accompanied by a more developed endomisium (Figure 2a).

Muscle fibers of some fascicles showed uniform size. In the remaining fascicles, however, the coexistence of small, intermediate and large muscle fibers was observed (Figure 2a). At this age, the number of adipocytes in the perimisium showed to be increased. Under histochemical reactions, the three main kinds of myofibers revealed distinct characteristics (2b e 2.c). This observations is compatible with a high degree of fiber differentiation.

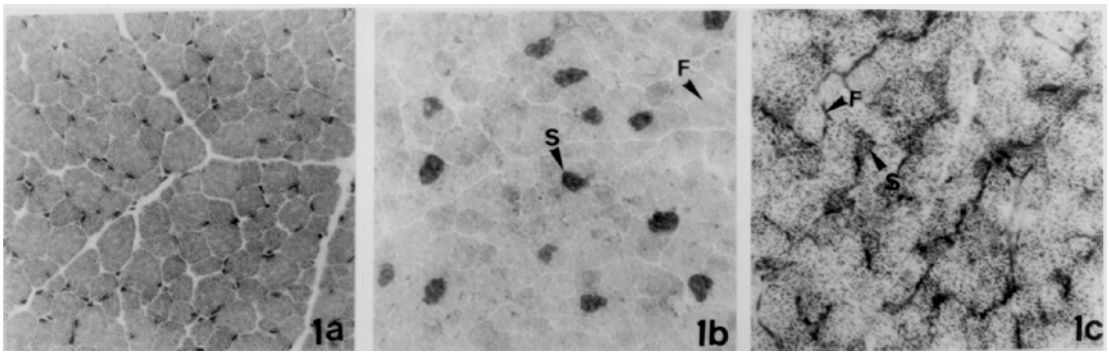

Figure 1. Transverse sections of *semitendinosus* muscle of lamb at birth: a - HE, X 50.; b - m-ATPase, pH 4.4, X 50. c - NADH-TR, X 50. Slow (S) and Fast (F) myofibers

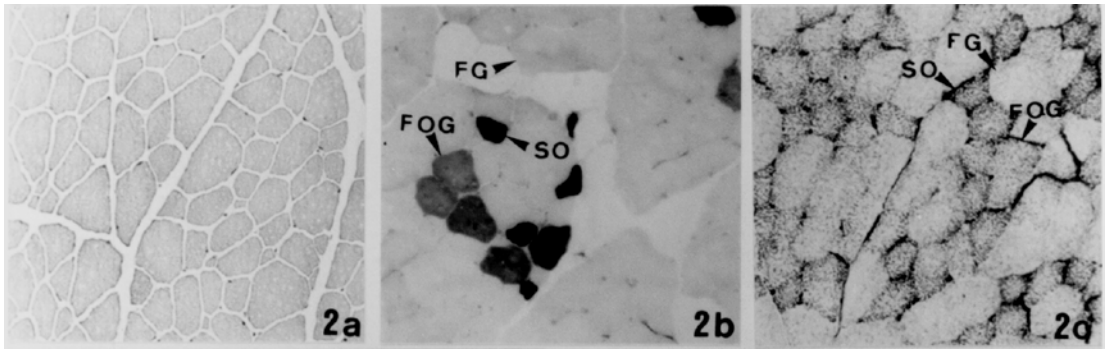

Figure 2. Transverse sections of *semitendinosus* muscle of lamb at the slaughter. a - HE, X 50.; b - m-ATPase, pH4.4, X 50; c - NADH-TR, X 50 Type Slow Oxidative (SO) myofiber, type Fast Oxidative Glycolytic (FOG) myofiber and type Fast Glycolytic (FG) myofiber

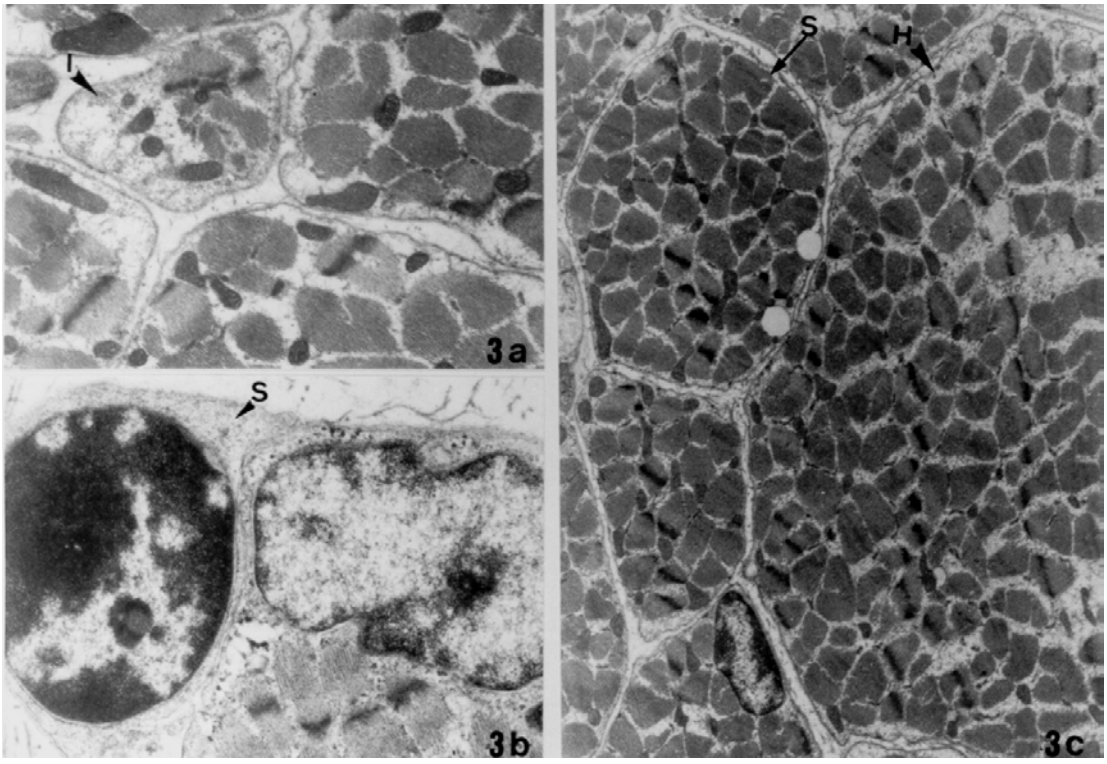

Figure 3. Electromicrographies of *semitendinosus* muscle of the lamb: a e b - at birth immature cells, (I), X 9.450, and satellite cells (S), X 17.000; c- at weaning, hypertrophic cells (H) and small cells (S), X 5.750

Despite the majority of muscle fibers presenting mature and hypertrophic aspect, the ultrastructural analysis revealed the presence of some small and undifferentiated fibers dispersed in the connective tissue and of satellite cells (Figure 3b). The relative high number of immature muscle fibers and of satellite cells at the slaughter age seems to indicate that the growing process of the animal is still active.

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