We have previously shown that during compensatory growth in bovine, muscle fibres are rapidly oriented towards glycolytic fibres (Brandstetter et al., Livest. Prod. Sci. 53 (1998) 25–36). Moreover, small fibres expressing developmental myosin heavy chain isoforms were observed, suggesting that a new population of muscle fibres is formed from recruitment of satellite cells. As high levels of insulin and triiodothyronine (T3) are restored during compensation, we studied the respective influence of both hormones on satellite cell proliferation and myogenesis.

Primary mononucleated cells, most likely satellite muscle cells, were isolated from cultivated muscle *Semitendinosus* explants: cells migrated out of the explants, proliferated and colonised culture dishes. After 1 week, myogenic cells were recovered by trypsinisation of subconfluent cultures. After preplating, cells were seeded at 10^4 cells/cm^2 in a mixture of DMEM and 10 % fetal calf serum in absence or presence of insulin (5, 10 and 50 µU/mL) or T3 (1, 3 and 5 ng/mL). On day 5, the serum concentration of the medium was lowered to 2 %. Cell proliferation was assessed by scoring the cell nuclei number after Giemsa staining. Differentiation was studied by the fusion index and by cyto-immunofluorescence analysis of myogenin, myosin heavy chains (MHC) and connectin.

Influence of insulin: an increase in cell density was observed in insulin-treated cultures. Stimulation of fusion by insulin was observed over all the time-course of culture, and was clearly apparent on day 11. On day 5, insulin treatment was associated with enhanced accumulation of myogenin, foetal and slow MHC, and connectin. These results indicate that insulin stimulates both satellite cell proliferation and differentiation in a dose-dependent manner. Interestingly enough, proliferation was highest at 50 µU insulin/mL (two-fold increase in nuclei number on day 5) while optimal differentiation was observed at 10 µU insulin/mL (three-fold increase in fusion index on day 11).

Influence of T3: we first tested the influence of a T3-depletion of the culture medium. No effect was apparent on cell density; fusion of satellite cells was delayed and decreased in T3-depleted cultures. In contrast to data reported on quail myoblasts (Marchal et al., Biol. Cell. 78 (1993) 191–197), a physiological dose of T3 (1 ng/mL) did not decrease satellite cell density. A moderate anti-proliferative T3 dose-dependent influence was observed at 3 and 5 ng/mL. Fusion was clearly apparent on day 3 in all T3-treated cultures and a dose-dependent stimulation of fusion was registered on day 11. In agreement with these morphological data, T3-treatment was associated on day 3 with a precocious detection of myogenin and rapid MHC. On day 5, an increase in foetal and slow MHC accumulation was observed in T3-treated cultures. Thus, T3 anticipated and enhanced the differentiation of bovine satellite cells in a dose-dependent manner.

This preliminary study indicates that insulin and T3 influence the proliferation and stimulate the differentiation of bovine satellite cells in primary culture. Both hormones have already been shown to regulate the acquisition of muscle fibre properties and muscle growth. Our data suggest that insulin and T3 could be implicated in the formation of new fibre from satellite cells during compensatory growth.

Communications no. 24

Comparison of in vitro proliferation and differentiation of bovine myoblasts from different genetic types. M.P. Duris, A. Delavaud, B. Picard (Équipe croissance musculaire, laboratoire croissance et métabolismes des herbivores, Inra, Theix, 63122 Saint-Genès-Champanelle, France).

It has been shown that the speed of growth selection of bovines is associated

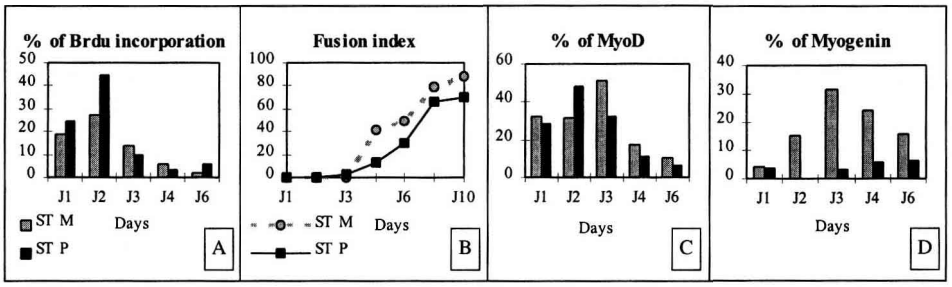

Figure 1. Differences in the mean number of nonatretic and atretic antral follicles between the two ovaries of calves treated with and GnRH agonist prior to pFSH superovulatory treatment. Asterisks within a column indicate differences in number of follicles between calves treated alike ($P < 0.005$).

with changes in muscle characteristics (Renand et al., Genet. Sel. Evol. 27 (1995) 287–298). Adult cattle with a high speed of growth (P) have larger muscles which contain more glycolytic and larger fibres than those with a low speed of growth (M). The aim of this work was to study the in vitro proliferation and differentiation phases of myoblasts from these two bovine genetic types during their embryonic development.

Primary cultures were obtained from myoblasts of *Semitendinosus* (ST) muscles of three 110-day-old charolais P and M foetuses. The proliferation was analysed after an immunological revelation with Bromodeoxyuridine (Brdu) incorporation. The differentiation was studied by the fusion index. Myogenic factors, MyoD and Myogenin, were quantified by antibody use.

The proliferation was qualitatively more important from day 1 and decreased more rapidly after day 2 in P myoblasts than in M ones (*figure 1A*). The fusion started after day 3 and increased intensively until day 10 in M myoblasts (*figure 1B*). In P myoblasts it started significantly at day 4 but with a lower intensity. A higher fusion index was observed in M than in P myoblasts. The myogenic factors study (*figures 1C* and *D*) showed that the percentage of MyoD enclosing cells decreased after day 2 in P cells and only after day 3 in M cells. This coincides with the fact that proliferation decreased less strongly in those cells. The percentage of Myogenin-positive cells was higher in M myoblasts, which was consistent with the higher fusion index in these cells. It led us to hypothesise that the myoblasts of P genotype present a delay of differentiation. A similar feature has already been observed in vitro for myoblasts from double-muscled bovines (Picard et al., BAM 8 (1998) 197–203).

Thus, muscle hypertrophy may have the same origin in P genotype and in double-muscled bovine. The analysis of myofibrillar proteins (desmin, titin and myosin heavy chains) during the differentiation phase will permit this hypothesis to be verified.

Communication no. 25

Circulating insulin-like growth factors (IGF-I and -II) and binding proteins in selected lines of chickens. C. Beccavin, B. Chevalier, M.J. Duclos (Station de recherches avicoles, Inra, Tours 37 380 Nouzilly, France)

Insulin-like growth factors (IGF-I and IGF-II) and their binding proteins (IGFBP) take part in the regulation of growth and body composition in a number of species