



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-muscling bovines (Picard et al., BAM 8 (1998) 197–203).

Thus, muscle hypertrophy may have the same origin in P genotype and in double-muscling 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

(Jones, Clemmons, *Endocrine Rev.* 16 (1995) 3–34). To test their importance in the chicken, two experimental models obtained by divergent selection were studied. Chickens with either a high (HG) or low (LG) growth rate (Ricard, *Ann. Genet. Sel. Anim.* 7 (1975) 427–443) were compared at 1 and 6 weeks of age. Fat and Lean chickens (Leclercq et al., *Br. Poult. Sci.* 21 (1980) 107–113) were compared at 9 weeks of age in the fed and the fasted state. Following their extraction from plasma or serum to eliminate IGF-BPs interference, IGF-I and IGF-II were measured by a radio-immunoassay with specific antibodies. Three circulating IGF-BPs with molecular weights of 28, 34 and 40–42 kDa (designated IGF-BP-28, -34 and -40, respectively) were detected by western ligand blotting and the intensity of each band was quantified using a Phosphorimager (Storm 840, Molecular Dynamics). IGF-I and IGF-II increased with age and were higher in HG compared to LG chickens. IGF-I and, to a lower extent IGF-II, decreased following a 48-h fasting period and altogether, IGF-I and IGF-II were higher in Fat compared to Lean chickens. Two of the IGF-BPs (-34 and -40) increased with age in growth-selected lines. IGF-BP-34 was higher in HG than LG chickens at 6 weeks of age. IGF-BP-28 and -34 (to a lower extent) increased in the fasted state in Fat and Lean chickens. In the fasted state, IGF-BP-34 was lower in Fat chickens. We conclude that, in the chicken, IGFs and IGF-BPs vary with age, nutritional state and genotype. IGFs and IGF-BP-34 and -40 increase with age. IGFs are down-regulated, and IGF-BP-28 and -34 are up-regulated by fasting, as in mammalian species. In two genotypes with different growth rates, higher IGFs levels correspond to higher growth rates, whereas in two genotypes which differ only for body composition, higher IGF levels correspond to an increased fat deposition.

Communication no. 26

Isolation of pure Glucagon Islets of Langerhans from Chicken Pancreas.

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In contrast to mammals, endocrine pancreas in birds is characterized by two basic islet-types: i) glucagon-rich or A-islets, ii) insulin-rich or B-islets (Bonner-Weir, Weir, *Gen. Comp. Endocrinol.* 38 (1979) 28–37). Until now, the physiology of these peculiar islets has remained totally unknown and information concerning the isolation of avian islets is lacking. This work aimed to obtain physiologically reactive chicken islets of Langerhans.

A procedure for selectively isolating A-islets from the chicken pancreas is described. It consists of ductal injection of collagenase P (Boehringer, 4 mg/g pancreas), enzymatic digestion, atraumatic dispersion of the digesta at an appropriate time and nylon mesh filtrations. A- and B-islet types were identified through immunohistochemistry and radioimmunological quantification of insulin and glucagon. Islets from the cranial half of the body of the pancreas were found to be almost pure (95 %) glucagon islets (mean content = 0.45 pmol glucagon and 0.02 pmol insulin per islet). The functional capabilities of isolated islets were analysed (within 2–5 h after isolation) by comparing hormone release from islets incubated in the presence of glucose. Increasing the glucose concentration from 14 to 42 mM decreased glucagon release (0.055 versus 0.040 pmol glucagon per islet per 30 min, respectively, $P < 0.05$), thus demonstrating that the α -cells responded to glucose after isolation. Sensitivity of α -cells to insulin content of the preparation is suggested. To date, pure glucagon islets have not been described in any species other than birds. This attractive new tool is now being further developed in our laboratory to provide better understanding of α -cell physiology.