Fish cells in primary culture : different models and applications

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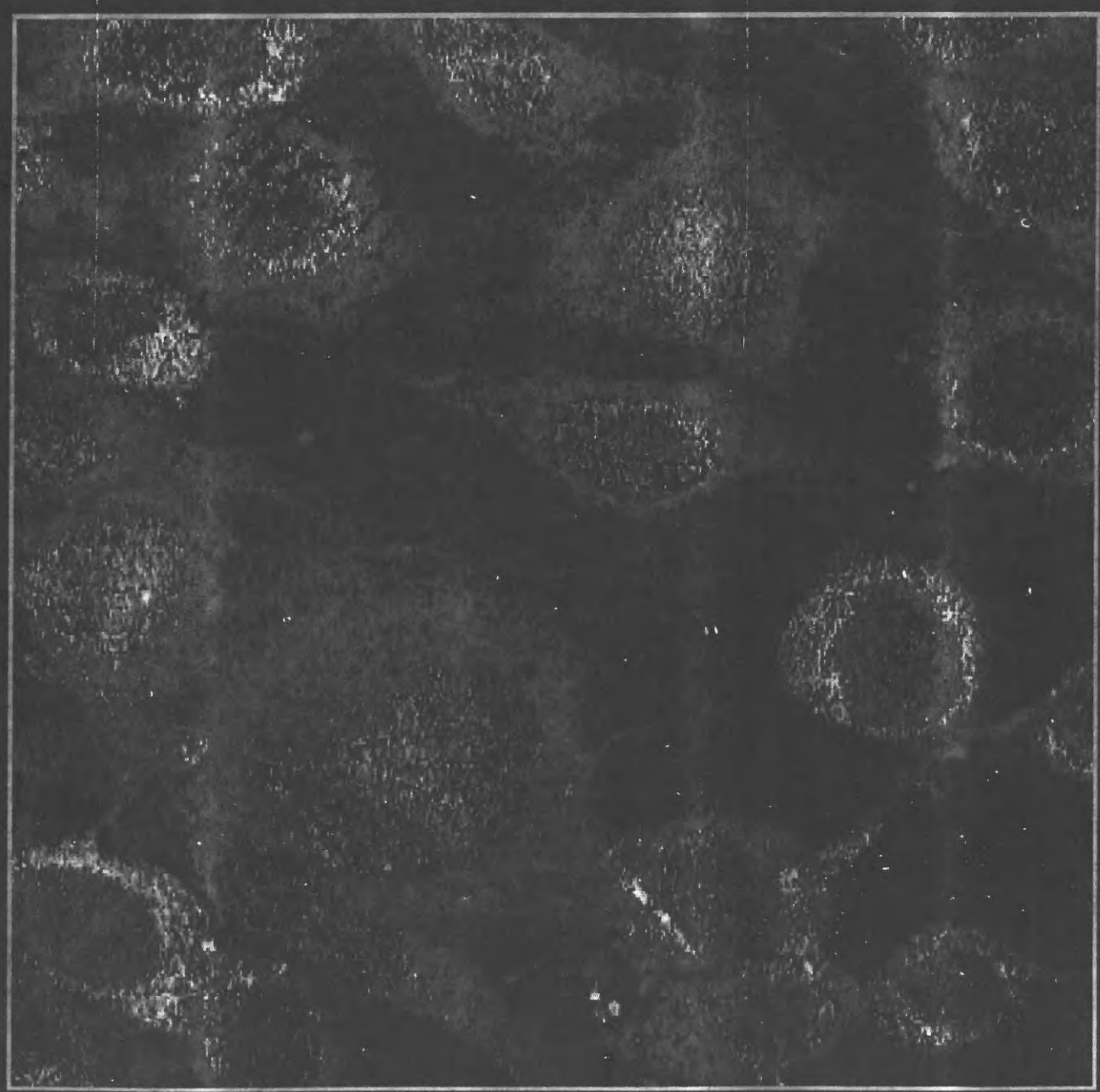

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FISH CELLS IN PRIMARY CULTURE: DIFFERENT MODELS AND APPLICATIONS.

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Studies in fish of different physiological functions (such as growth, reproduction, osmoregulation) at a cellular level led us to develop primary cell cultures from different organs including pituitary, testis, ovaries, muscles and gills. These cell culture techniques appeared to be powerful tools allowing us to analyze in vitro molecular mechanisms associated with a particular physiological function and ,thus, to explore different aspects of cell functions such as differentiation, secretory activity, intracellular pH regulation or cell cooperativity. In this presentation, we shall briefly describe these cell culture system and illustrate the potential of these model systems.

Primary cultures of rainbow trout granulosa cells were developped, using the possibility to isolate oocyte and their granulosa cells from the other ovarian components. Optimal factors for granulosa cells isolation and culture were settled following their ability to secrete a specific steroid inducing oocyte maturation. Such a cell culture model was used to study the hormonal regulation of steroid synthesis during the final steps of oogenesis. It is also proposed for testing xenobiotic effects on the female reproductive physiology.

From rainbow trout testis, enriched populations of spermatogonia and primary spermatocytes were prepared by centrifugal elutriation and further cultured for 3 days. This culture system allowed us to demonstrate in vitro a direct mitogenic effect of IGF-I and IGF-II on these testis cells.

Primary cultures of total pituitary cells were set up and used for studying the regulation of the pituitary function. We focused our interest on the regulation of gonadotrophin, growth hormone and prolactin. Furthermore, this culture system was used to compare biological activity of different fish GnRH or mammalian LHRH analogs on gonadotrophin secretion in rainbow trout. This allowed us to select in vitro the most potent molecule which was further be tested in vivo for its ability to induce ovulation.

The culture of muscle cells would provide a good model to study the mechanisms involved in the processes and in the regulation of skeletal muscle development. The different events of myogenesis were observed: active proliferation, early differentiation (expression of desmin and alignement), fusion into multinucleated myotubes and maturation (expression and organization of contractile protein into myofibrils). The analysis of the factors involved in the induction of satellite cells and in the control of their proliferation and differentiation is now in progress.

Primary cultures of trout gill cells were undertaken in order to precise the mechanisms of pH regulation. After few days in culture, two types of chloride cells were revealed using mithochondrial probes (mitochondria rich cells and mitochondria poor cells). Monoclonal antibodies were produced in order to identify the growing cells in primary culture. Preliminary results suggest the existence of a protons extrusion mechanism depending on sodium (Na^+/H^+antiporter). Further studies are undertaken to precise the mechanisms involved in the pH regulation of these cells.

Beyond the originality and the availability of fish cells as models for studying cells functions, these sudies provide significant knowledges for others applications such as, control of organism biology for aquaculture purpose (spawning induction, gamete production, quality of growth...) or ecotoxicology (in vitro evaluation of ecotoxicity on validated models).