Development of primary cell cultures from sea urchin gonads

Silvia MERCURIO, Cristiano DI BENEDETTO, Michela SUGNI, M. Daniela CANDIA CARNEVALI

Department of Bioliogy, University of Milan, Via Celoria, 26 - 20133 Milano, Italy

Corresponding author: Silvia Mercurio: silvia.mercurio@unimi.it

Keywords: *sea urchin, gonads, primary cell culture*

The Mediterranean sea urchin *Paracentrotus lividus* is one of the favorite and most used experimental model in developmental biology. Taking into account the advantages and possible applications of the *in vitro* studies, the first attempts to develop primary cell cultures from gonads of this species were carried out. A detailed histological analysis was performed in order to characterize the cellular phenotypes present in fresh cultures. As observed in the *in vivo* model, only germinal cells and nutritive phagocytes were observed in male and female gonad cell cultures. Three different modified culture media were tested: Leibovitz-15 (L-15), Medium 199 (M199) and Minimum Essential Medium Eagle (MEM). The most suitable medium was determined by a detailed analysis of cell morphology and viability and, on the basis of these results, L-15 appeared to be the best medium for echinoid gonad cells. Various substrates were also tested. Gonad cells adhered only on poly-l-lysine substrate while we did not find any improvements in terms of cell adhesion using mammalian collagen, gelatin and sea urchin collagen substrates. In order to stimulate cell growth and survival L-15 medium was supplemented with growth factors or embryo extracts. In particular, the effects of inactivated standard Fetal Calf Serum (FCS) and of an originally developed "Pluteus Estract" at different concentrations were analyzed and compared. This preliminary study suggests that it is possible to develop primary cell cultures from sea urchin gonads and maintain these cells under *in vitro* conditions for more than one month. Overall, our findings represent an important starting point for the establishment of proliferative primary cell culture from *P. lividus* gonads.