Culturing of embryonic stem cells isolated from blastula stage eggs of Atlantic cod, *Gadus morhua.*

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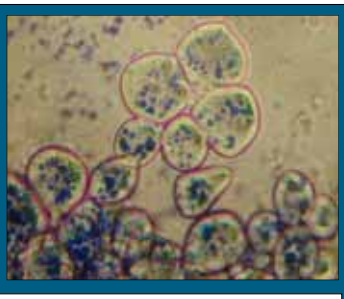
Following fertilization, all vertebrate embryos consists of pluripotent cells. Pluripotency is defined as the embryonic stem cells (ES cells) ability to generate all cell types of the embryo; endoderm, ectoderm and mesoderm (Niwa, H., 2007). Culturing embryonic stem cells from cod is a step in understanding the molecular interactions underlying cod specific development. The necessity of identifying stage specific cod markers and markers that identifies specific cod cell types are mandatory before establishing cellular differentiation models or stage specific in vitro systems with a broad range of applications.

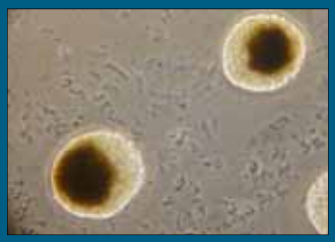
Stages of development:

- 1 DPF:cleavage and early blastula
- 1.5 DPF: Blastula
- 2 DPF: Early Gastrula, some eggs were still inn late blastula
- 3 DPF: Gastrula and 50% Epiboly
- 6 DPF: Segmentation period

Materials and Methods: **Fertilized eggs were prepared for cell culture within 24-26 h (DPF1-1.5) following fertilization. The eggs were crushed and cells were filtered through a nylon mesh before washing. The cells were seeded on gelatin coated cell culture slides and incubated in a normal atmosphere incubator at 10°C. The culture medium was DMEM with high glucose and HEPES supplemented with FBS, Glutamax, MEM, Sodium Selenite, Sodium Puryvate Solution and Mercaptoethanol.**

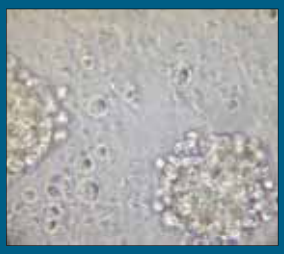


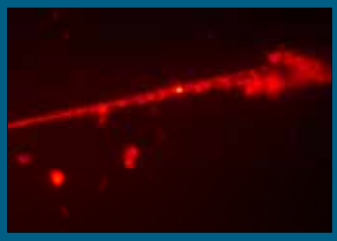
ES cells freshly isolated from cod eggs (1.5 dpf) (left). ES cells attached to gelatin and coloured for better visualization, right All photos: 40x10xs



Unidentified (due to lack of functioning cell markers) spontaneous differentiated cod ES cells: floating embryonic cell clusters (left), and a "fibroblast" like cell type (right)

Cod ES cells cultured on low adherence dishes in the presence of medium and all-trans retinoic acid form Embryonic Bodies (EB)





In an approach of inducing ES cell differentiation the cells were cultured on fibronectin coated cell culture slides in medium enriched with insulin like growth factor II, platelet derived growth factor, nerve growth factor, epidermal growth factor, N-2 supplementation and B-27 supplementation. For immunofluorescence staining a mouse anti-neuronal class III beta tubulin antibody combined with Texas red dye conjugated secondary antibody was used to verify the neuronal like cell type.

Conclusions:

Cod ES cells *in vitro* show common features for all ES cells, spontaneous differentiation and the ability to form embryonic bodies following retinoic acid treatment.

The ES cells could be directed to differentiate upon relevant treatment which is promising for the evaluation of optimal protocols for cell differentiation models.

We need to identify several cod specific markers as a tool for the further development of cod ES model systems

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