

Subject Area: Advanced Methods in Biotechnology and Biodiversity

Subject: Cyto- and histochemical analysis of embryonic animal tissues

Level: PhD

Year: I-IV

Semester: 1- 2

Speciality: N/A

Status: Facultative

ECTS: 3

Department(s): Department of Animal Histology and Embryology

Cooperating Department:

Form of teaching (Number of hours; Form of assessment: Exam or Credit)

<i>Lectures</i>	<i>Seminars/Conversatoria</i>	<i>Practicals</i>	<i>Total</i>
4 hrs		26 hrs	30 hrs

Staff:

SUBJECT COORDINATOR: Prof. Piotr Świątek Ph.D.

LECTURE/CONVERSATORIA: Magdalena Rost-Roszkowska Ph.D.

PRACTICALS: Izabela Poprawa Ph.D., Magdalena Rost-Roszkowska Ph.D., Weronika Rupik Ph.D., Prof. Piotr Świątek Ph.D.

Contents:

LECTURES:

Instruction to cyto- and immunocytochemistry: immunoenzyme, immunofluorescence and EM immunogold labelling.

PRACTICALS:

Principles of animal dissection. Tissue preservation by freezing and chemical methods (i.e. formalin, glutaraldehyde). Cutting of fixed and unfixed frozen sections on a cryostat and mounting sections on microscopic slides. Embedding fixed tissues in paraffin, Epon, Histocryl and LR White resins. Whole mount analysis of fixed tissues. Demonstration of dehydrogenases and phosphatases using tetrazolium salts, metal precipitation techniques and azo-dye methods for sections. Detection of cytoskeletal elements as microfilaments (rhodamine-conjugated phalloidin staining) and microtubules (immunostaining with anti β -tubulin). Using of nuclear dyes – DAPI and IP. The elements of immunocytochemistry: immunofluorescence and immunoenzyme direct and indirect techniques. Immuno EM (electron microscopy) technique – labelling with antibodies conjugated with colloid gold. Using of TUNEL assay and caspase 3 antibodies for apoptosis detection. Using Mn SOD and Cu/Zn SOD antibodies for detection of superoxide dismutase (SOD). Analysis of the obtained probes with the use of light, fluorescence and TEM microscopes.

As material we use animal tissues connected with development as ovaries, testes, developing amphibian eggs ect.

Methods and forms of teaching:

Lectures with projection of slides. Practical training in using cyto- and histochemical methods in developmental biology.

Requirements:

Literature:

1. Hayat M.A. 2003. Microscopy, Immunohistochemistry, and Antigen Retrieval Methods: For Light and Electron Microscopy. Kluwer Academic/Plenum Publishers.
2. Cuello A.C. 1993. Immunohistochemistry II. Wiley&Sons.
3. Bancroft J.D., Gamble M. 2007. Theory and Practice of Histological techniques. Sixth edition. Churchill Livingstone.
4. McIntosh J.R. 2007. Methods in cell biology. Vol. 79. Cellular Electron Microscopy. Elsevier.

Remarks (if necessary):