The role of activin A in the regulation of preimplantation development of mammalian embryo

Embryogenesis of the mouse, which is considered as a model mammal, takes place in the female reproductive tracts. Preimplantation period of mouse development extends from fertilization to implantation of the embryo in uterus. Over this period, the cells (blastomeres), which emerge as a result of cleavage of the zygote, gradually differentiate and arrange to form the fluid-filled blastocyst. It consists of two separate cell lineages displaying different properties and different developmental significance: Trophectoderm (TE) and inner cell mass (ICM). The trophectoderm is a layer of outer cells that forms the wall of the blastocyst and during further development contributes exclusively to the embryonic part of the placenta. The inner cell mass, which is composed of pluripotent cells occupying one pole of the blastocyst interior, shortly before implantation differentiates into two distinct subpopulations of cells. Primitive endoderm (PE) emerges as a monolayer of cells on the surface of ICM and after implantation contributes to the endoderm layer of fetal membrane, the yolk sac. Deeper cells of ICM comprise pluripotent epiblast (EPI) that is the material for the future fetus body.

It is well known that the preimplantation mammalian embryo, in contrast to the embryos of other vertebrates as well as invertebrates, is characterized by a large degree of plasticity. In response to experimental manipulations, *i.e.*, removal, addition or rearrangement of cells, it can adapt and follow normal course of development culminating in birth of the animal. The cells of the embryo communicate with each other, "sense" this excess or deficiency and use different strategies to adapt and successfully proceed with further development. This communication between cells relies on sending and transmitting information in the form of secreted proteins, which, by binding to their receptors on the surface of neighboring cells, stimulate their differentiation into a specific cell lineage. The plasticity of preimplantation embryo has been applied for preimplantation genetic diagnosis allowing to test human embryos before transferring them to the mother's uterus. It also helped to explain the reasons of monozygotic pregnancies, resulting from division of one fertilized egg into two separate embryos. Despite many lines of evidence for the regulative nature of mammalian embryos, the mechanisms responsible for this phenomenon have not yet been fully elucidated. One of the signaling pathways potentially involved in the regulation of mouse embryo development is activin A, a protein belonging to the TGFβ (*transforming growth factor beta*) family. The role of the pathway activated by this protein in the postimplantation development of the mouse embryo is relatively well known. However, despite the presence of its components in the preimplantation period of development, its exact significance is still elusive.

The aim of the proposed project is to investigate whether activin A plays a role in the specification and segregation of cell lineages in the mammalian embryo, guaranteeing the formation of blastocyst ready for implantation. To this end, we plan to examine the consequences of the total lack of a gene encoding activin A. We also plan to examine whether cells of the embryo communicate via activin A signaling and whether Fgf4/MAPK (*fibroblast growth factor/mitogen-activated protein kinase*) and activin A pathways act synergistically to regulate appropriate proportions of cells contributing to the cell lineages. To achieve this goal, we want to experimentally disrupt the interactions between cells by microinjection of embryonic stem cells, which produce and secrete both of these proteins, into 8-cell mouse embryos and their culture until the blastocyst stage in medium supplemented with specific inhibitors blocking one or both of these pathways. We expect that misregulation of this precise cell-cell communication will result in disorders in cell lineages formation and failure of development.

Since recent molecular studies have revealed unexpected differences between different mammalian species and raised concerns regarding the extent, to which we can extrapolate research from mouse (regarded as a model mammal) to humans, we decided to extend our research to non-rodent animal model (rabbit).

The results of our experiments will allow not only to broaden the knowledge regarding the mechanisms controlling the fate of cells in the mammalian embryo, underlying its regulative capabilities, but they can also provide the basis for optimization of human assisted reproductive techniques and technology of transgenic animals generation.