Objective of the project

Exploration of the processes responsible for germ cell specification and development is crucial for understanding causes of human infertility which is a serious medical problem worldwide. The NANOS3 protein is critical for the human germ cell development, since *NANOS3* gene mutations cause female infertility, manifesting with the absence of germ cells. NANOS3 is an RNA-binding protein which is expressed starting at the time of the germ cell specification and early development at the 12th day of the human embryo development. Therefore, the general objective of this project is to get insight into NANOS3 processes it regulates and which are altered upon the specific NANOS3 Glu120Lys mutation causing female infertility. We expect that by comparing functioning of the normal NANOS3 with the mutated NANOS3, we will be able to select pathways that are the most important for specification/early stages of human germ cell development.

Research to be carried out

Knowing that NANOS3 has ability to bind RNA molecules, we plan to identify NANOS3-bound RNAs, given that such RNAs were not studied yet. However, considering the ethical issues, we cannot perform such studies in humans and much less in human embryos in whom those processes take place. Therefore, we will use a model mimicking specification and early stages of the human germ cell development. Our model of choice is based on *in vitro* growing human embryonic stem cells (hESCs) which we will differentiate toward the germ line. We will apply cutting edge methodologies for a global identification of RNA molecules that the normal NANOS3 protein binds to during germ cells specification and the first days of development. We will check which among them are specific for a particular time point of the germline development. Importantly, we will perform that experiment for both, the normal and the mutated NANOS3 to understand the molecular mechanisms which are altered by the female infertility causing mutation. Finally, the most interesting NANOS3-bound RNAs will be further tested for their functional dependence on the NANOS3 presence using several approaches. The choice of approaches to be used will be dependent on the function of the proteins these NANOS3 RNA targets encode.

Justification of the research and expected results

Infertility affects approximately 15% of couples worldwide. Genetic abnormalities account for 15%–30% of infertility. We expect that this study will enable to get a global insight into processes responsible for a proper specification and early germ cell development, which are controlled by NANOS3 protein. Severity of the patients infertility characteristics (absence of germ cells in ovaries) indicates that those processes are crucial for human fertility. Within this project we plan to identify RNAs which are involved in those processes. The results we will obtain within this project may be used in the future to elaborate genetic diagnosis tools for couples which cannot conceive and are seeking to undergo *in vitro* fertilization. We expect that our findings will also be of value for future therapies of infertility.