To improve the livestock production, high reproductive efficiency is one of the most important factors. Efficient *in vitro* embryo production is currently a major objective for livestock industries and at the same time one of the leading biotechnological method facilitating reproductive efficiency in cows. The heterogeneity of oocytes collected by the ovum pick up method or from ovaries of slaughtered females, still remains a challenge for *in vitro* embryo production success and limits the rate of embryo development. To generate and select embryos of high developmental potential, the selection of the highest quality oocytes is fundamental. Therefore, searching for the appropriate molecular oocyte quality markers and gene markers of embryonic implantation ability and developmental competence, as the alternative for the widely used subjective morphological assessments, is the matter of interest for many research groups.

Mitochondria play important roles in oocyte maturation and subsequent embryonic development, since they are responsible for producing most of the energy in the form of adenosine triphosphate (ATP). The presence of mitochondria in oocytes are required to support early embryonic development since glycolysis is limited during oocyte maturation and early preimplantation embryo development, until the blastocyst stage. In cow, a reduced mitochondrial DNA in poor quality oocytes, selected based on morphology, was detected. For the latter aim, cumulus cells collected after *in vitro* fertilization might facilitate a non-invasive analysis, as mitochondrial DNA content of those cells correlates with that of the corresponding oocyte, and a higher mitochondrial DNA copy number in cumulus cells has been linked to better embryo quality in pigs, human and cows. Therefore, in the present study we plan to apply the strategy of utilizing the mitochondrial DNA content and its quality as a marker for identification of the quality of oocytes, since cumulus cells are closely coupled to the oocyte through paracrine and intercellular communication systems and play major roles in acquisition of oocyte developmental competence.

In our project we aim to find some molecular markers for the quality of the bovine oocytes using, a well-defined in the literature, bovine model for investigation of oocyte developmental competence based on the sexual maturity of the oocyte donor cows. The developmental ability to blastocyst formation from fertilized oocytes obtained from calves is significantly lower than that obtained from cows. It has been also documented that transfer of blastocysts obtained from calf oocytes resulted in a lower pregnancy rate than that achieved with cow oocytes. Despite the evidence of biochemical defects in prepubertal oocytes such as altered protein synthesis, aberrant energy metabolism, reduced activity of maturation promoting factors, relatively less is known about the molecular characteristics of such oocytes, manifested as the level of differences in mRNA transcript profiles between oocytes collected from prepubertal versus adult animals. It remains unclear what is the difference in the expression of defined gene program between embryos derived from prepubertal and pubertal animals at crucial stages of their preimplantation development. This expression of the defined gene program remains in the area of particular interest of this project and includes the factors involved in mitochondrial function and markers of blastocyst implantation ability and developmental competence.

Talking all above into consideration, the scientific objective of the proposed research project is to investigate whether mitochondrial and metabolic adjustments during bovine *in vitro* early embryo development reflect the quality of oocytes.

The present study makes an effort to determine: [1] the relation of the mitochondrial DNA damage at different stages of the early embryo development with the quality of bovine oocytes, [2] the correlation between the level of mitochondrial DNA damage and metabolic changes in the cumulus cells and the quality of bovine oocytes as well as [3] the influence of the level of mitochondrial DNA damage and metabolic modifications in the cumulus cells on pregnancy success after transfer of corresponding blastocysts and changes in their transcriptome.

In the presented project we plan to determine the mitochondrial and metabolic changes during bovine early embryo development *in vitro*. For this purpose, we will evaluate mitochondrial DNA damage and embryo quality in the *in vitro* produced bovine embryos at different stages of their early, preimplantation development derived from good and poor quality oocytes as well as in the cumulus cells surrounding the fertilized oocyte after *in vitro* fertilization. We will also determine transcriptional differences in genes involved in mitochondrial function and quality markers in the preimplantation embryos derived from poor and good quality oocytes as well as in the cumulus cells surrounding the fertilized oocyte after *in vitro* fertilization. In the cumulus cells surrounding the fertilized oocyte after *in vitro* fertilization derived from good and poor quality oocytes we will assess the ATP content, activity of enzymes involved in glucose metabolism as well as quantify energy metabolites in the culture medium. In order to determine the fertilization outcome of good and poor quality oocytes, after *in vitro* fertilization we will determine sperm penetration and the extend of zona pellucida hardening. Finally, the evaluation of the transcriptomic changes in blastocysts and pregnancy rates after transfer of the blastocysts obtained *in vitro* from oocytes with granulosa cells with high and low level of mitochondrial DNA damage derived from good and poor quality oocytes, will be performed by the single cell RNA seq.