The main goal of this project is the complex characterization of secretive properties and differentiation capabilities of human granulosa cells (GCs) and cumulus cells (CCs). This aim will be realized by estimation of their proliferation/migration speed, in real time, using three types of culture media (main medium type, osteoblast/chondroblast differentiation inducting medium and spent embryonic culture conditioned medium). Additionally, an attempt to determine the interactions between GCs and CCs, kept in a co-culture in the media listed above, will be made. A special focus will be placed to estimation of human embryo culture conditioned medium effect on the differentiation potential of studied cells. Characterisation of GCs' and CCs' secretive properties will be based on their proteomic/metabolomic analysis, as well as study of spent culture medium contents.

To fulfil the aims of the study, following methods will be used:

- GC and CC cultures and co-culture in 3D hydrogel systems
- Analysis of GC, CC and GC+CC migration/proliferation speeds in different culture media, in real time, using RTCA (The Real Time Cell Analyzer)
- Determination of known genetic markers in GC, CC and GC+CC culture samples, using immunofluorescence approach
- Proteomic/metabolomic analysis of protein contents of GCs and CCs (from separate cultures and co-cultures), spent culture medium, as well as spent embryonic culture medium, using mass spectrometry (NanoLC-MALDI-TOF/TOF MS/MS)
- Transcriptomic analysis of differentiated cells, coming from GC, CC and GC+CC cultures, using different molecular techniques (expression microarrays, RT-qPCR)

Physiology of GCs and CCs is relatively well known in *in vivo* conditions. However, the extensive change in the properties of those cells, when they are subjected to *in vitro* culture conditions, is not yet well discovered. It is suggested, that GCs have stem-like properties, being able to transdifferentiate into other cell types when properly induced. It is not known if the CCs also possess these characteristics, but as both of those cell types are located in close proximity and share a common origin, this probability seem very likely. Hence, complex transcriptomic and metabolomic characteristic of CCs, as well as determination of their proliferation/migration potential is one of the aims of this project.

In the recent years, more and more scientific interest is focused on the used of conditioned media, obtained from specific cell cultures. The use of spent human embryonic culture medium seems to be one of the most promising approaches. This is mostly because this medium contains substances/factors/metabolites released by the embryo during its growth. The embryos, while being prepared for the transfer (during IVF procedures) are kept *in vitro* until they reach blastocyst stage. The remnant medium is treated as a disposable material. Hence, it seems logical to study its contents, to potentially use it as a supplement in other cell cultures. As for now, its chemical composition, as well as influence over cell cultures was not successfully determined. It is possible, that this remnant material that, until now, lacked proper research will have some kind of influence on the tempo and direction of GC and CC differentiation.

This project also aims to obtain stable and defined lines of chondrocytes and osteoblasts, that could be later used in regenerative medicine and transplantology. Additionally, identification of spent embryonic culture medium contents could determine its therapeutic potential and analyse the possibility of its use, as a supplement, during stem cell differentiation into different lineages.

Innovative approaches in this project are: use of spent embryonic cultures medium, GC and CC co-culture with the use of membrane inserts, with evaluation of their potential for differentiation into other cell types, as well as its interdisciplinarity and the possibility to apply the resulting cells in regenerative and reconstructive medicine.