

PROJECT SUMMARY

Reproduction is undoubtedly one of the largest branches of the equine veterinary medicine throughout the world. The knowledge in this field has had enormously increased, however, despite such progress reproductive efficiency of mares remained unchanged. One of the largest problems in mares' reproduction is early pregnancy loss that is before day 40 of pregnancy. This time correlates with the formation of the placenta. However, immune factors such as specific cells which may influence placentation in mares and establishment of the maternal tolerance towards the fetus remain unknown.

Hence, the aim of the project is to determine the immune mechanisms facilitating placentation in this species. In particular, we want to characterize the components of the innate and adaptive immunity which play a key role in the establishment of maternal immune tolerance to the fetus and their involvement in the development of the placenta.

We hypothesize that natural killer (NK) cells which are the component of the innate immunity may be involved in the control of the interdigitation between the allantochorion (fetal part of the placenta) and the endometrium (maternal part of the placenta) during the trophoblast movement. We speculate that transcriptome of NK cells will change during the process of placentation and will differ between the time of trophoblast invasion and the establishment of the firm attachment. We hypothesize that these cells together with regulatory T cells (Tregs) which represent adaptive immunity may facilitate the tolerance towards paternal antigens once the movement of the trophoblast into the endometrium is completed and placenta is formed.

In details in the proposed project we want to:

- 1). Optimize a methodology to isolate cells from the endometrium suitable for single cell RNAsequencing (scRNAseq)
- 2) Compare the subtypes of cells in the endometrium at the beginning and following completion of placentation
- 3). Determine the phenotype of the uterine NK cells in the endometrium during and after placentation
- 4). Determine the phenotype of the regulatory T cells (Tregs) during placentation.
- 5). Identify the signals derived by the allantochorion which represents the fetus that recruit NK cells and Tregs into the endometrium at the time of placentation.

To investigate these objectives samples of the endometrium and allantochorion will be collected during the formation of the placenta (day 33-35 of pregnancy) and once the fusion of trophoblast with the endometrium is completed (day 42-45 of pregnancy). A novel technique which allows identifying single cells within the collected sample that is scRNAseq will be used to characterize the phenotype of endometrial cells including NK cells and Tregs. Because there is no protocol for isolation of the equine endometrial cells of a quality suitable for the scRNAseq analysis the first step will be the optimization of this method with the use of abattoir material. Once the conditions of the cells isolation are established, scRNAseq will be performed on samples collected from experimental mares. In the next step transcriptome of the allantochorion by RNA sequencing will be performed what will allow identifying immunomodulatory factors secreted by the conceptus which might attract/influence the NK cells and Tregs in the endometrium. Influence of these secreted molecules on maternal NK cells and Tregs will be further confirmed in in vitro studies. This approach ensures detailed characterization of the immune processes during placentation in mares.

Physiology of early pregnancy in mares is not only a scientific and veterinary challenge. Related to that period reproductive disorders such as EPL are associated with substantial economic implications for horse owners. This project has a novel character and obtained results will significantly expand the knowledge in the field of equine reproductive immunology. Moreover, due to similarities between equine and human pregnancy such as presence of the invasive trophoblast and secretion of the chorionic gonadotropin mare can be treated as a model organism to study some aspects of the human pregnancy.

Furthermore, cells of the adaptive and innate immune response are not fully characterized in horses. Knowledge of the phenotype of the uNK cells as well as Tregs which are involved in the development of the tolerance is limited. We believe that results of the proposed project will increase the knowledge not only in field of equine reproduction but also equine immunology and possibly help to develop new immunotherapies, for example in allergic related diseases, making our research the more valuable.