

The first successful reports of *in vitro* maturation (IVM) of equine oocytes and their use for the production of embryos date back to the 1980s. During this period, research on the IVM process, *in vitro* fertilization (IVF) or *in vitro* embryo culture (IVC) in other animal species, such as cattle, sheep and pigs, was at an advanced level, responding to the basic scientific questions, but also finding application in the commercial industry. The main factor limiting the application and development of assisted reproductive technologies (ART) in equines is the low availability of research material. The clearly lower efficiency of the vital oocyte recovery rate in horses is caused by the specific structure of the ovary, which leads to problems with inducing superovulation. Moreover, the post mortem oocyte recovery rate is limited due to the difficult access to slaughter material. For these reasons, ART in horses is still unsatisfactory, which can be confirmed by the inability to perform classic IVF and significantly lower rates of oocyte *in vitro* maturation, reaching only approximately 50%, and even 90% in ruminants.

The IVM process takes place in culture media, the task of which is to provide oocytes with conditions as close to physiological as possible. However, the media used in equines is prepared based on the components of the media used in other species, as the specific requirements of the equine oocytes during these processes are still largely unknown.

Despite the use of various supplements to the culture media, it is still unknown which of them interact with gametes and embryos. However, it is known that the intercellular communication processes within the ovary and the follicle microenvironment are the basic mechanisms that enable oocytes to mature. Oocyte development involves the processes by which the gamete undergoes cytoplasmic, nuclear and molecular maturation. During all stages of oocyte maturation, cellular communication between the oocyte and the surrounding granulosa cells is essential, as well as a process known as cumulus expansion. In recent years, it has been shown that signal transduction between cells can be mediated by extracellular vesicles (EVs), which have the ability to deliver molecular signals to target cells. EVs are an important component of body fluids such as ovarian follicular fluid, making them widely studied for their role in cell-to-cell communication and for their effects on ART.

Ovarian follicular fluid EVs (ffEVs) has been shown to contain bioactive components such as miRNAs that affect genes related to metabolism, reproduction and endocrine function, which are of great importance for a successful pregnancy. Recent reports indicate that ffEVs affect the expression of selected cumulus cell genes as well as the expansion of cumulus cells in cattle and mice. However, evidence showing a physiological effect of ffEVs on cumulus cell function in domestic horses during IVM has not yet been demonstrated. The research hypothesis is that ffEVs can affect the physiology and morphology of the cumulus-oocyte complex and induce changes in the cumulus transcriptome, which may help create a favorable microenvironment for oocyte maturation, fertilization and early embryonic development. The aim of the project is to evaluate the effect of ffEVs from small (<20 mm) and large (> 30 mm) follicles on viability, expansion and transcriptome modulation of the cumulus cells.

The mare's ovaries, obtained from a local slaughterhouse, will be used for the research. EVs will be isolated from the ovarian follicular fluid by ultracentrifugation. In the next stage, oocytes obtained from ovarian follicles will be matured *in vitro* with the use of ffEVs supplementation. Measurements of the diameter of the cumulus-oocyte complexes before and after IVM will be performed to evaluate the effect of ffEVs on the cumulus expansion. After the culture, the oocytes will be stained to evaluate the viability of the cumulus cells. In addition, next-generation sequencing and bioinformatics analysis will be performed to assess transcriptome-level changes in cumulus cells exposed to ffEVs.

Supplementation of media at various stages of assisted reproduction with ffEVs may be a perfect complement to the physiological needs of gametes and embryos in the early stages of development. Understanding the influence of ffEVs supplementation on the transcriptome alterations of cumulus cells may bring us closer to understanding the mechanisms by which EVs affect recipient cells. Therefore, we aim to expand the basic knowledge of the role of ffEVs in the significant processes of cumulus expansion and oocyte *in vitro* maturation.