Analysis of Cytoplasmic Movement Velocity as a tool for assessment of actomyosin cytoskeleton quality in mammalian oocytes

Infertility has grown beyond the dystopian science-fiction issue, becoming a global crisis. Increasingly late parenthood and lifestyle changes of the third millennium can, however, be countered now with the help of assisted reproductive technologies (ART). *In vitro* fertilization (IVF) has become one of the most important procedures for treating infertility. Despite recent advances, increasing the success rate to even 55% in patients younger than 35, the efficiency of IVF is still astonishingly low for women over 40 years old, reaching less than 18%. ART is also increasingly often used in veterinary medicine for breeding livestock and endangered animals preservation. However, although over a million cow embryos a year are produced, horse or sheep embryos production remains but a 0.25% fraction of this success at best. The ART industry still requires development and procedure optimization, thus scientists are urged to explore novel approaches to oocytes and embryos selection procedures and enhancement of reproductive outcomes. The success of assisted reproductive technologies is often linked to the quality of the chosen oocytes, therefore methods for oocyte quality assessment are in demand, both in assisted reproduction of humans and domestic or endangered animals. We would like to contribute to the progress of ART and test a simple, non-invasive method for oocyte quality assessment, based on oocytes' actomyosin-mediated cytoskeletal properties.

The cytoskeleton plays a key role in the segregation of chromosomes, cellular division, and organelle trafficking, each of which is important for cell cycle progression. Actomyosin is a protein complex formed by molecules of actin and myosin, which is responsible for cell's contractibility. A vast network of regulatory proteins interacts with actomyosin, controlling cell's function, shape and elasticity. The actomyosin cytoskeleton can be damaged due to ageing, both maternal (i.e. the age of the mother) and postovulatory (i.e. the extended period between the ovulation and fertilization), as well as ART-related procedures like cryopreservation, and possibly *in vitro* maturation of oocytes. This cytoskeletal impairment could be in part caused by damaging protein modifications, resulting from oxidative stress. The quality of the cytoskeleton can be mirrored by oocyte or embryo biomechanical properties, which can be tested by the analysis of the cytoplasmic movement velocity (CMV). No one, to our knowledge, has used this technique to assess the quality of unfertilized mature oocytes. Our preliminary data suggest that cytoplasmic dynamics is hindered in postovulatory and maternally aged mouse oocytes, compared to freshly ovulated oocytes collected from young mice.

The proposed project aims to verify the following hypotheses: that (1) CMV analysis reflects the quality of the actomyosin cytoskeleton in oocytes, including those from developmentally compromised pools (i.e. subjected to cryopreservation, *in vitro* maturation or ageing); (2) CMV analysis may be applied to assess the quality of mature oocytes prior to their fertilization and predict its outcomes.

In our experiments, we will use modern cell biology techniques, such as live-cell imaging, biomechanics measurements or fluorescent labelling of cytoskeletal proteins. We will correlate CMV in oocytes with their development success after fertilization. We will also examine how oocytes' diverse background (e.g. ageing) and ART-related treatment (cryopreservation, *in vitro* maturation) affects the structure of actomyosin cytoskeleton and biomechanical properties of oocytes, including CMV. Finally, we will, for the first time, investigate the impact of oxidative damage of proteins on oocyte biomechanics. This research could provide a basis for further research on damaged protein disposal mechanisms and advance the repertoire of oocyte selection techniques used in reproductive and veterinary medicine, contributing to the optimization of ART procedures.