Biomechanical properties of trophectoderm as a marker of mammalian embryo quality.

Approximately 15% of couples in reproductive age suffer from fertility problems and *in vitro* fertilization (IVF) is one of the most popular methods of treating infertility. Despite recent great advancement in IVF procedures, their efficiency, measured as a live birth rate per cycle, reaches 50% in young patients but is several times lower in older patients. Apart from clinical use, IVF plays also an essential role in livestock breeding programmes and preservation of endangered species. To increase the efficiency of this procedure, only the most viable embryos should be selected for transfer to mothers. This, in turn, puts a constant pressure on scientists and physicians to develop novel, reliable methods of evaluating embryonic developmental potential. In the current project, we plan to examine whether biomechanical properties of trophectoderm (TE), a tissue participating on behalf of the embryo in implantation to the uterus, can serve as predictors of the developmental capabilities of mammalian embryos. We wish also to study a relationship between biomechanical properties of TE and keratin cytoskeleton, recently reported as a key regulator of TE cell differentiation. Finally, as ageing, both maternal (related to female's age) and postovulatory (related to the time between ovulation and fertilization), strongly affects the efficiency of IVF procedure, we will examine its effect on biomechanical properties of TE and on their usability as predictors of the embryo quality. We will conduct our experiments on mouse embryos, which are commonly used as a model in developmental and reproductive biology.

TE cortical tension will be assessed by micropipette aspiration method, in which pressure required to deforming TE is measured, and then correlated with the embryo's ability to implant, examined first by in an *in vitro* assay, and then *in vivo*. This will allow us to create an algorithm that predicts embryo's ability to implant. Next, we will examine the relationship between TE cortical tension and data obtained by time-lapse imaging (TLI): (i) blastocyst expansion dynamics, and (ii) velocity of cytoplasmic movement in TE cells. This will allow us to find the TLI-derived parameters that most reliably reflect TE biomechanical state; they will be also correlated with the embryo's ability to implant, and we will attempt to formulate algorithms that predict embryo's ability to implant. The most promising algorithms will be tested for embryo-safety and validated on an independent data set. Moreover, to identify biomechanical parameters useful in detecting keratin cytoskeletal defects in embryos, we will examine TE biomechanical properties in embryos with experimentally decreased or increased amount of keratin 8 and 18 proteins. Finally, we will assess TE biomechanical properties in embryos obtained from oocytes subjected to maternal or postovulatory ageing and examine whether biomechanical parameters can serve as quality predictors for such embryos.

To our knowledge, no one has tried yet to use the assessment of TE biomechanical properties by micropipette aspiration or by analysis of cytoplasmic movement to evaluate the mammalian embryo developmental potential. There have been attempts to correlate dynamics of blastocyst expansion with embryo’s developmental ability, but the results are ambiguous and it has not been properly verified whether the parameters analysed reflect TE biomechanical properties. Moreover, we will attempt to find biomechanical parameters that reflect dysfunctional keratin cytoskeleton in embryos. This is an interesting and clinically relevant task, as keratin cytoskeleton defects cannot be easily detected by standard analysis of embryos’ divisions and keratin-defective embryos fail in postimplantation development. Furthermore, we wish to examine the usability of the biomechanical parameters in embryos obtained from maternally or postovulatory aged oocytes. Ageing impairs oocytes', and in consequence embryos', physiology in a heterogeneous way. Therefore it is extremely important for the IVF efficiency to select reliably those embryos that maintain the highest quality. Taking into account the above-mentioned arguments, we believe that our research dedicated to the application of TE biomechanical properties as indicators of embryo developmental potential will be an important contribution to embryology and reproductive biology, and in future, it may help to improve protocols of embryo assessment in clinical and veterinary practice.