Postovulatory and Maternal Aging Do Not Affect the Biomechanical Properties of Trophectodermal Cells in Mouse Embryos

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As literature data indicate, postovulatory aging - resulting from the extended time between ovulation and fertilization, and maternal aging - related to the mother's age, are among the most important causes of low oocyte quality, which in turn results in lower developmental potential. It has been reported that different types of aging may affect fertilizability, the ability to form a blastocyst, and postimplantation embryo development. However, there is no data on the impact of aging on the functionality of the trophectoderm, which is crucial for the proper implantation process. Some of the most important determinants of trophectoderm functionality are its biomechanical properties, which are reflected, among others, as the velocity of cytoplasmic movement. In our studies, we checked the impact of both maternal and *in vitro* postovulatory aging on the velocity of cytoplasmic movement in trophectoderm cells of mouse blastocysts. Using time-lapse imaging and the Particle Image Velocimetry method, we analysed cytoplasmic movements in trophectodermal cells of the following groups: 1) control – blastocysts obtained from *in vitro* fertilization (IVF) of freshly ovulated oocytes from young females, 2) postovulatory aging – blastocysts obtained from IVF of oocytes (obtained from young females) subjected to nine-hour-long *in vitro* aging, and 3) maternal aging – blastocysts obtained from IVF of freshly ovulated oocytes isolated from old females. First, we found that the velocity of cytoplasmic movement was significantly higher in polar than in mural trophectoderm cells: 10.14±3.89 nm/s and 6.61±2.32 nm/s in control embryos, 10.94±2.42 nm/s and 6.50±1.80 nm/s in postovulatory aging group, 10.29±3.13 nm/s and 7.05±2.36 nm/s in maternal aging group in polar and mural trophectoderm cells, respectively. However, we did not observe the effect of maternal and *in vitro* postovulatory aging on the velocity of cytoplasmic movement both in the mural and polar trophectoderm cells. Because in our previous experiments, we discovered that the cytoplasmic velocity depends on the keratin cytoskeleton, we checked the impact of maternal aging on mRNA levels for keratin 8/18 (the most abundant in preimplantation embryos) in mouse blastocysts. We found that the mRNA levels for keratin8/18 are not changed by maternal aging, which is consistent with the lack of differences in cytoplasmic velocity. To sum up, the adverse effects of maternal and *in vitro* postovulatory aging on the embryos' development most likely do not result from changes in the biomechanical properties of the trophectodermal cells.

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