Extension of Development (ED) of Mouse Blastocyst leads to Activation of Steroidogenesis

Hafsa Gulzar[1,2]; Richard Musson[1,2]; Joanna Depciuch[3]; Michał Kobiałka[1]; Simona Bisogno[1]; Maria F. Heber[1], Grażyna E. Ptak[1]

[1] Malopolska Centre of Biotechnology, Jagiellonian University in Kraków, Poland; [2]Doctoral School of Exact and Natural Sciences, Jagiellonian University in Krakow, Poland;[3]Institute of Nuclear Physics, Polish Academy of Sciences, Kraków, Poland

It is believed that preimplantation mouse embryo is not capable of steroidogenesis, unlike species with an extended period of preimplantation development (ruminants, pigs and horses). We previously demonstrated in mice that when implantation is prevented by ovariectomy, the blastocyst switches from carbohydrate to lipid metabolism at 8 days post coitum (dpc). This leads us to hypothesise that ***mouse blastocyst is able to synthesize steroids once its preimplantation development is extended.***

Naturally mated C57BL/6 female mice were ovariectomised at 3 dpc to remove the source of oestradiol and prevent embryo implantation. Then, embryos were collected at 4, 6, 8, 10 and 12 dpc (control: E4; experimental groups: E6-8-10-12). Embryo collection media, considered as a representation of embryonic content (bearing in mind nonactive uterus of ovariectomised mice) were processed for exosome isolation. In collected (n>10/group) blastocysts and exosomes, lipid profile was analysed using Fourier Transform Infrared (FTIR) and Raman spectroscopies. Moreover, gene expression and cellular structures such as lipid droplets, autophagosomes and mitochondria (where the first step of steroidogenesis takes place) were analysed in blastocysts by mRNA sequencing and transmission electron microscopy (TEM). Finally, blastocyst growth was evaluated by fluorescence immunolabeling and cross-sectional measurement of semi-thin sections.

FTIR analysis of blastocysts demonstrated temporal increase of cholesterol, oxidised cholesterol and polyunsaturated fatty acids (PUFAs) absorbance that are recognised modulators of steroidogenesis (0.227, 0.381, 0.231 for E8 vs. 0.216, 0.338, 0.218, for E4, respectively, $p<0.05$) during ED. Exosomes analysis show that total lipid level increases at E10 (FTIR: 0.164 vs. 0.125, $p<0.05$; Raman: 0.067 vs. 0.006, $p<0.05$); lipid peroxidation level decreases during ED (15.213, 4.142, 5.963, 10.304, 4.736 for E4, E6, E8, E10 and E12, respectively, $p<0.05$) and PUFAs level increases at E10 (0.021 vs 0.016, $p<0.05$); one-way ANOVA with Tukey's post hoc test. Moreover, the expression of genes regulating cholesterol metabolism (*Apol6, Scap, Npc2* and *Cyb5a*) and steroidogenesis (*Dhrs11, Hsd17b, Hsd3b*, *Cyp4, Cyp26a* and *Cyp27a*) was activated during ED. Interestingly, *Cyp27a* expression indicates the generation of 27-hydroxycholesterol that is considered to be an ancestral oestrogen. Sequencing data are expressed as log2 fold change, differentially expressed genes were considered when false discovery rate<0.05. TEM analysis revealed indications of steroidogenesis changes in cell composition during ED, such as autophagy, demonstrated by the presence of lysosomal bodies and autophagosomes, and degradation of lipid droplets and changes in the profile of mitochondria (i.e. the organelle where the biosynthesis of steroid hormones occurs). Mean number of blastocyst cells at E8 and E4 was 60±2.5 vs. 51.2±2.5 cells±SEM, $p=0.04$, for trophectoderm, and 19±0.5 vs. 21±1.8 cells±SEM for inner cell mass. There was a progressive increase of the cross-sectional area of blastocysts (2785.298<2915.040<3760.608<5041.595μm²) at E4, E6, E8 and E12, respectively.

We provide the evidence of steroidogenic activity in mouse blastocysts during ED. The observation of naturally occurring and experimentally induced implantation delay in mice demonstrates that ED blastocysts are capable of implantation and development to term. Thus, activation of steroidogenesis

may be an evolutionary-conserved mechanism that facilitates implantation under specific conditions such as ED, in all mammals.

This work was supported by the National Science Centre, Poland GA no. 2019/35/B/NZ4/03547 and 2021/41/B/NZ3/03507.

Key words: cholesterol, steroidogenesis, blastocyst size, lipid droplets, autophagy