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Maternal metabolic disorders and early embryonic loss: pathways to bridge the gap between bovine embryo quality and endometrial receptivity.

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The pre-implantation embryo is very sensitive to perturbations in its micro-environment and, therefore, a tight regulation of the embryonic milieu is essential. Such an environment is not assured in females suffering metabolic disorders. Our previous data show that altered nutrient abundance in the embryonic micro-environment results in suboptimal embryonic cell differentiation patterns. Here, we hypothesize that suboptimal nutrient conditions during embryo culture can affect the blastocyst's capacity to participate in the first maternal-embryonic interactions.

Earliest preimplantation phases of embryo development were studied as 'window' for nutrient sensitive manipulations. Embryos (4 repeats; 890 zygotes) were cultured during the first 4 days after fertilization under distinct nutrient conditions: [control] based on serum-free SOF medium; [HIGHGLUC] with 3.5mM glucose; [LOWAA] with 10% lower amino acid concentrations as presented in control. At morula stage, embryos were transferred on monolayers of epithelial endometrial cells (BEEC), in SOF medium with 5% serum, till D8 p.i.. In D8 blastocysts, qRT-PCR was used to screen mRNA expression of genes involved in nutrient sensing, pluripotency and differentiation. Differently expressed genes (DEG) were identified using (mixed model) ANOVA. Transcriptomes of BEEC exposed to the distinct groups of embryos were sequenced and data were normalized and using the EdgeR package.

Blastocysts originating from HIGHGLUC morulae displayed a tendency for increased transcript levels of *PDK1* (P=0.075), a key gene in nutrient sensing regulation. Also a down-regulated expression of the pluripotency marker, *OCT4 (P=0.002),* was observed compared to controls*.* Transcriptome reaction of BEEC exposed to the HIGHGLUC embryos was rather limited. Only 27 genes were differently expressed (DEG), of which 20 down- and 7 up-regulated in BEEC exposed to HIGHGLUC embryos compared to control embryos (*Padj*<0.1). The enriched genes could be associated with endoplasmic reticulum activities, whereas genes involved in cell-cell signalling pathways displayed down-regulated expression. Blastocysts from LOWAA conditions showed tendencies (P≤0.1) for decreased transcript levels of *SIRT1*, *mTOR*, *GLUT1* and *LDHA*, all genes involved in mTOR pathways. Furthermore, a down-regulated mRNA expression of *OCT4* (P<0.0001) and *SOX2* (P<0.1)*,* both key genes involved in blastomere pluripotency, was observed. Blastomere differentiation markers, such as *ITGB5* (P<0.05) and *CTNN1* (P≤0.1) displayed decreased transcript levels in LOWAA blastocysts compared to controls. Transcriptome data from BEEC exposed to LOWAA embryos, revealed 120 DEG compared to BEEC exposed to controls (*Padj*<0.1). Here, 63 of the 120 DEG were down- and 57 were up-regulated in the LOWAA condition. Up-regulated genes could be assigned to transcription regulation and down-regulated genes concerned pathways inhibiting Notch (proliferation) and innate immune responses.

Overall, these data show that suboptimal metabolite conditions during the first 4 days of embryo culture prompt nutrient sensing programs and impact on resultant blastocyst cell proliferation and differentiation pathways. Furthermore, BEEC genes were differently regulated when placed in contact with the three distinct groups of embryos. Decreased integrin (*ITGB5)* gene expression in LOWAA blastocysts, and subsequent down-regulated expression of cell adhesion factors (i.e. *LAMB1*, *CCDC80*) in the allied BEEC, points towards the importance of ligand-ligand interaction and the key role of integrins for the first maternal-embryonic interactions.