32nd Scientific meeting of the European Embryo Transfer Association (AETE) in Barcelona, Spain from the 9th to the 10th of September 2016

Maternal impact of metabolic diseases: Effect of nutrient sensing pathways on developmental and differentiation programs in the bovine embryo.

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Maternal metabolic disorders affect reproductive physiology, leading to a disappointing fertility. A correct proliferation and differentiation process of the inner cell mass (ICM) versus trophectoderm (TE) embryonic cells is a prerequisite for successful embryo-endometrial cell interactions upon embryo arrival in the uterus. Recent cancer cell studies revealed that regulation of cell differentiation occurs via nutrient-sensing mechanisms. In this context, glucose and amino acids are upstream factors regulating the mTOR driven nutrient-sensing pathways, coupling metabolite abundance to cell growth and differentiation.

Here, the earliest preimplantation stages of embryo development were studied as 'window' for nutrient sensitive manipulations. Embryos were cultured during the first 4 days after fertilization under distinct nutrient conditions: [C1] CONTROL based on the SOF medium; [C2] HIGH GLUCOSE using 3.5mM glucose; [C3] LOW AMINO ACID containing only 10% of amino acid concentrations as presented in C1. At morula stage, embryos were transferred to a routine SOF medium, with 5% serum, till D8 p.i.. Blastocysts from 4 replicates were immune-stained for cell differentiation (ICM/TE ratio) and apoptotic cell index (ACI) using CDX, Casp3 and Hoechst. Embryo development was analyzed using binary logistic regression and other parameters via (mixed model) ANOVA.

Cleavage and blastocyst rates were similar for all groups ($P>0.05$). However, the capacity of cleaved zygotes to reach blastocyst stage tended to drop after embryo culture till morula stage under C2 and C3 conditions (29.2% and 30.8%, respectively) compared to the C1 group (37.2%) ($P<0.1$). No differences in total cell numbers were observed when comparing treatment groups. Nevertheless, a significant shift in cell lineage commitment was noticed; C1 displayed higher ICM/TE ratios (0.65±0.04) compared to C2 and C3 blastocysts (0.41±0.02 and 0.49±0.02, respectively; $P<0.02$). Furthermore, a twice as high ACI was noted for the blastocysts from C2 (0.30±0.04) and C3 (0.35±0.03) compared to C1 (0.15±0.02) ($P<0.001$). More specifically, the ACI of the ICM fraction was drastically increased in C2 and C3 (0.58±0.09 and 0.55±0.06; respectively) compared to C1 (0.11±0.01) blastocysts ($P<0.001$). The latter can contribute to the observed drop in ICM/TE ratios in C2 and C3 blastocysts. In addition, a significant increase in TE ACI was noticed in C2 (0.14±0.02) and C3 (0.23±0.03) compared to C1 (0.10±0.01) blastocysts ($P<0.05$).

In conclusion, a bovine preimplantation embryo responds to nutrient abundance in its micro-environment resulting in suboptimal trophoblast cell arrangements. The latter might jeopardize first maternal-embryonic interactions and thereby pregnancy can be threatened in women suffering metabolic disorders.

Key words: nutrient, embryo, differentiation