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

THE EFFECTS OF HYPO- AND HYPERGLYCEMIA DURING LIPOLYSIS-LIKE CONDITIONS ON BOVINE OOCYTE PHYSIOLOGY

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Next to elevated non-esterified fatty acid (NEFA) concentrations, lipolytic metabolic conditions can be associated with hypo- and hyperglycemia. Previous research has shown that in the presence of high NEFAs, hypoglycemia (LO GLUC) during IVM hampers embryo development to a greater extend as compared to hyper- (HI GLUC) and normoglycemic conditions. Blastocyst metabolism and carbohydrate- and oxidative stress related gene expression were not affected, but blastocysts from LO- and HI GLUC exposed oocytes showed a higher degree of apoptosis. As a consequence we aimed to study the effects of hypo- and hyperglycemia in presence of elevated NEFAs on oocyte metabolism, apoptosis and reactive oxygen species generation (ROS). Hereto bovine cumulus oocyte complexes (COCs) were routinely matured (1 COC/10µL medium) during 24h under different NEFA and GLUC levels: 1) physiological NEFA (72µM palmitic, stearic, oleic acid) and routine IVM GLUC (5.5mM) (=CONT), 2) pathophysiological NEFA (420µM) and routine GLUC (=HI NEFA), 3) HI NEFA+HI GLUC (10mM) and 4) HI NEFA+LO GLUC (2.8mM). Initial and conditioned medium was sampled (4 repeats) and analyzed for glucose and lactate concentrations. After IVM, all COCs were fixed and stained with caspase-3 and HOECHST to determine apoptosis (n=182, 3 repeats) or denuded and stained for intracellular ROS during 30min using H_2DCFDA (n=79, 3 repeats). H_2DCFDA fluorescence intensity was quantified using ImageJ and COC apoptosis was classified as: <25%, 25-75% and >75% cumulus cell apoptosis. All data were compared between 4 treatments using a mixed model ANOVA and Bonferroni post-hoc (IBM SPSS Statistics 20). Means ± SEM are presented. COCs exposed to HI NEFA+HI GLUC consume significantly less glucose (485 ± 63 pmol/COC/h) compared with the other treatments (mean of 894 ± 35 pmol/COC/h). HI NEFA+HI GLUC and HI NEFA+LO GLUC exposed COCs produced significantly less lactate (1738 ± 192 and 1848 ± 51 pmol/COC/h, respectively) than CONT and HI NEFA exposed COCs (3573 ± 212 and 3494 ± 289 pmol/COC/h). In addition, LACT/GLUC ratio was significantly lower in all treated COCs compared with CONT, with the lowest LACT/GLUC ratio in HI NEFA+LO GLUC exposed COCs indicating a shift of glucose towards pathways other than glycolysis. No differences were observed in COC apoptosis between treatments. Oocyte ROS was significantly higher in HI NEFA+HI GLUC exposed oocytes (15.85 ± 1.87) compared with HI NEFA+LO GLUC (13.06 ± 1.28) and HI NEFA oocytes (12.06 ± 1.11). In conclusion, lipolytic conditions with or without glycemic perturbations influence the oocyte's glucose and lactate metabolism. Whereas hypoglycemia in the presence of elevated NEFAs hampers embryo development the most, high GLUC exposed oocytes suffer from increased intracellular ROS. This could not be substantiated by increased cumulus cell apoptosis.

Key words: NEFA, glucose, oocyte