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Effect of different culture conditions on bovine embryos derived from metabolically-compromised oocytes

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Metabolic disorders e.g. obesity lead to elevated saturated (stearic; SA, palmitic; PA) and mono-unsaturated (oleic; OA) free fatty acids (FFAs) in serum and follicular fluid. Exposure of maturing oocytes to these FFAs, particularly to PA, hampers embryo development. Supplementation of embryo culture media with Insulin-Transferrin-Selenium (ITS) or serum is used to enhance embryo production, however the effect of such enrichment on development of metabolically compromised oocytes has not been investigated. Here, bovine oocytes (n=3737) were exposed to either 1) pathophysiological high PA, SA and OA concentrations (150, 75, 200 µM, respectively; HFA); or 2) high PA, basal SA and basal OA (150, 28 and 21 µM; HPA); compared to 3) basal PA, SA and OA (23, 28, 21 µM; BASAL) as a physiological control. Zygotes were cultured in SOF medium containing 1) BSA only or supplemented with 2) ITS or with 3) serum. Cleavage (48h) and blastocyst rates (day 7 (D7) and D8 post insemination) were recorded. D8 blastocysts were analyzed for apoptotic cell indexes (ACI) (caspase-3 immunostaining), embryo metabolism (glucose consumption and lactate production), or mRNA expression of genes involved in ER unfolded protein responses (UPR^{er}) (*Atf4, Atf6*), oxidative stress (*SOD2, GPx, CAT*) mitochondrial UPR (*HSPE1, HSPD1*) and mitochondrial biogenesis (*TFAM*). Categorical and numerical data were analysed using binary logistic regression and ANOVA, respectively, and were Bonferroni corrected. Cleavage rate was significantly ($P<0.05$) reduced in HPA embryos compared with BASAL when cultured in BSA. However, ITS or Serum in culture alleviated this negative effect. Compared with BASAL, HPA exposed oocytes showed significant lower D7 and D8 blastocyst rates after culture in BSA and Serum, but not in ITS containing SOF medium. Within the PA-treated group, ITS significantly increased D7 and 8 blastocyst rates compared with BSA. HFA did not have significant effects on development under all IVC conditions. For embryo quality, ACI was not different among BASAL, HFA and HPA groups in BSA culture. Surprisingly, supplementation of ITS during IVC significantly increased ACI of HPA and HFA embryos compared to BASAL ($P<0.05$). Serum supplementation also increased ACI of HPA embryos compared with HFA and BASAL ($P<0.05$). Regardless of IVM treatment, embryos cultured in Serum showed increased lactate/2glucose ratio compared with BSA and ITS, confirming the reported preference for Warburg metabolism. In contrast, HPA-derived embryos cultured in ITS or Serum had significantly lower lactate/2glucose ratio compared to BASAL and HFA. At the blastocyst transcriptomic level, HPA increased *HSP60* expression compared to BASAL when cultured in BSA, indicating activation of mitochondrial stress responses. ITS and Serum alleviated this increase in *HSP60.* In conclusion, enrichment of embryo culture media with ITS or serum can improve developmental competence of oocytes after maturation in lipotoxic conditions. However, the surviving blastocysts exhibit higher apoptosis and altered metabolism indicating inferior quality.