Changes in liver metabolism in mouse offspring born after in vitro embryo culture

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During in vitro culture (IVC) the embryo can be exposed to oxidative stress. We recently show that IVC induces oxidative changes in lipid droplets in preimplantation embryo, and that lipid changes are maintained during fetal development. Because the liver plays a central role in lipid metabolism, here we hypothesized that IVC can cause changes in hepatic lipids in offspring, which can predispose to steatosis and liver disease. C57BL6 embryos collected one day following mating of 8 pairs of 3-4 months old mice were collected and cultured in vitro in KSOM medium using standard procedures. Total of 95 blastocysts were then transferred to pseudopregnant recipients for further development (8-12 embryos/female). Four months old male offspring obtained following IVC and control offspring obtained following natural mating were randomly selected for liver analysis (n=8/group). Collected liver was homogenized and subjected to evaluation of lipid peroxidation, antioxidative mechanism status and proteome. Statistical significance was assessed by Mann-Whitney test, data are described as Mean±SEM.

High level of lipid peroxidation product, malondialdehyde (MDA) (27.63±9.03 vs. 18.99±1.93 nmol/mg of protein, p=0.03) measured by Thiobarbituric acid reactive substance (TBARs) assay was demonstrated in livers of IVC offspring. Furthermore, pro-oxidative status of liver from IVC offspring was shown by an increased levels of oxidative stress markers: GSH (1.11±0.2 vs 0.2±0.06 nmol/mg of protein, p=0.002); GSSG (0.37±0.05 vs 0.16±0.04 nmol/mg of protein, p=0.0093) and high superoxide dismutase activity (SOD) (4.0±0.7 vs. 2.2±0.2 U/mg of protein, p=0.03) evaluated by colorimetry assay. Tandem Mass Tag (TMT) proteomic analysis revealed 88 differentially expressed proteins (DEPs) in IVC group (34 upregulated and 54 downregulated DEPs). Enrichment analysis indicated that most of up-regulated proteins are involved in lipid metabolism pathways, such as, fatty acid oxidation, PPAR signalling pathway, beta-oxidation, alcoholic liver disease, lipid accumulation, and hepatic steatosis. Most of 54 down regulated DEPs were involved in oxidative stress and redox pathway, glutathione metabolism, fatty acid beta-oxidation, synthesis of polyunsaturated fatty acids and other metabolic pathways. Altogether, liver of males born after embryo culture of mouse embryos is characterized by oxidative damage of lipids and altered expression of proteins regulating lipid metabolism.

During early stages of development the liver is sensitive to changes in the environment. Our study indicates that in vitro environment can induce embryonic lipid damage and subsequent development of the liver. Consequent to embryonic lipid damage deregulation of hepatic lipid homeostasis may predispose to steatosis and liver disease later in life.

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