Mono (2-ethyllexyl) phthalate induces transcriptomic and proteomic alterations in bovine oocytes.

Dorit Kalo, Anais Vitorino Carvalho, Catherine Archilla, Véronique Duranthon, Marco Moroldo, Yishai Levin, Meital Kupervaser, Yoav Smith, Zvi Roth

Proceedings of the 35th Annual Meeting of the European Embryo Transfer Association (AETE); Murcia, Spain, September 12th and 14th, 2019. Abstracts.

A169E Embryology, developmental biology and physiology of reproduction

Mono (2-ethylhexyl) phthalate induces transcriptomic and proteomic alterations in bovine oocytes

Dorit Kalo[1,2], Anais Vitorino Carvalho[3], Catherine Archilla[3], Veronique Duranthon[3], Marco Moroldo[4], Yishai Levin[5], Meital Kupervaser[5], Yoav Smith[6], Zvi Roth[1,2]

[1]Department of Animal Science, Robert H.Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Israel; [2]Center of Excellence in Agriculture and Environmental Health, The Hebrew University of Jerusalem, Israel; [3]UMR BDR, INRA, ENVA, Université Paris Saclay, Jouy-en-Josas 78350, France; [4]Animal Biological Resources for Integrative and Digital Genomics Facilities, Jouy-en-Josas 78350, France; [5]The de Botton Institute for Protein Profiling, The Nancy and Stephen Grand Israel National Center for Personalized Medicine, Weizmann Institute of Science, Rehovot 76100, Israel; [6]The Bioinformatics Unit, Faculty of Medicine, the Hebrew University, Jerusalem 91120, Israel.

Keywords: phthalates, oocyte developmental competence, transcriptome.

Phthalates are plasticizers, used in a variety of industrial plastics. Di(2-ethylhexyl) phthalate (DEHP), the most commonly used plasticizer, and its main metabolite, mono(2-ethylhexyl) phthalate (MEHP), are known reproductive toxicants. A residual concentration of MEHP (~20 nM) has been found in follicular fluid aspirated from IVF-treated women and DEHP-treated cows. Previously we have reported that exposure of oocyte during maturation to MEHP impairs nuclear maturation, reduces clevage and blastocyst formation rates. However, the effect of 20 nM MEHP on the transcriptomic profile of oocytes and their derived blastocyts is not entirely clear. Bovine oocytes were in-vitro matured with or without 20 nM MEHP for 22 h. At the end of maturation, they were collected for transcriptomic (by microarray; n = 20 per sample; 4 replicates) and proteomic (n = 200 per group) analyses to examine a possible direct effect of MEHP on the oocyte transcriptomic and proteomic profiles. The remaining oocytes were in-vitro fertilized and embryonic development was recorded 42–44 h and 7 days postfertilization. Blastocysts were also collected for microarray analysis (n = 10 per sample; 4 replicates). Transcriptomic data were analyzed using Partek Genomics Suite software. Control probes were removed; signals were log2 transformed followed by interslide quantile normalization. Genes were considered differentially expressed if the *P*-value by one-way ANOVA was lower than 0.05 and absolute fold change was 1.5 between the control and MEHP-treated group. Proteomic raw data were imported into Expressionist® followed by Mascot software. Data were searched against the bovine sequences from UniProtKB. Proteins were considered differentially expressed at a fold change of ±1.5 with at least 2 unique peptides. Oocyte transcriptome analysis revealed MEHP-induced alterations in the expression of 456 genes. The differentially expressed genes were associated with actin cytoskeleton (n = 47 genes; e.g., *ACTG1*), metabolic pathway (n = 43) including oxidative phosphorylation (n = 12; e.g., *ND5*), oocyte maturation (n = 9; e.g., *PIK3CA*), and embryonic development (n = 14; e.g., *SOX10, NOTCH*); 191 proteins were affected by MEHP in mature oocytes, associated with methylation and acetylation (n = 51), metabolic pathway (n = 33) including mitochondrial oxidative phosphorylation (n = 7; e.g., ATP5E), and cytoskeleton structure (n = 32; e.g., ACTN1, EGFR). In control vs. MEHP-derived blastocysts, 290 genes were differentially expressed, associated with transcription process, cytoskeleton regulation and metabolic pathway; 9 of these genes were impaired in both oocytes exposed to MEHP (i.e., direct effect) and blastocysts developed from those oocytes (i.e., carryover effect). The study explores, for the first time, the risk associated with exposing oocytes to relevant MEHP concentrations (i.e., those found in the follicular fluid) to the maternal transcripts. Although it was the oocytes that were exposed to MEHP, alterations carried over to the blastocyst stage, following embryonic genome activation, implying that these embryos are of low quality.