EFFECTS OF OCHRATOXINAON NUCLEAR AND CYTOPLASMIC MATURATION OF OOCYTES OBTAINED FROM PREPUBERTAL LAMBS

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OchratoxinA (OTA) is a major mycotoxin produced by several species of Aspergillus and Penicillium fungi and has been reported as an ubiquitous natural contaminant found in food and feed[1].OTA plays reprotoxic, embryotoxic and teratogenic as well as nephrotoxic, neurotoxic, immunotoxic and carcinogenic activity as reported in either laboratory or farm animals [2]. Major mechanisms of action include inhibition of protein synthesis,toxic effect on mitochondrial (mt) function and calcium homeostasis with consequent oxidative stress, apoptosis induction and DNA adduct formation [2]. The aim of the present study was to evaluate the effects of OTA on nuclear and cytoplasmic maturation of oocytes from pre-pubertal lambs.Cumulus-oocyte complexes (COCs) were recovered at local slaughterhouses from the ovaries of prepubertal lambs (less than 6 months of age). During in vitro maturation (IVM) [3], COCs were exposed to 10 µM OTA, a concentration reported as effectivein a previous study in the mouse[4]. Control conditions were: vehicle controls (IVM medium with 1% methanol) and standard controls (IVM medium without vehicle). After IVM and the removal of cumulus cells, the oocytes were stained with MitoTracker Orange CMTM Ros, 2′,7′-dichlorodihydrofluorescein diacetate and Hoechst 33258 and fixed in 2% paraformaldehyde solution in PBS. Metaphase II oocytes were analyzed by laser scanning confocal microscopy for assessing their cytoplasmic maturation indicated by mt distribution pattern [2]. Data were analysed by Chi-square test and differences were statistically significant when $P<0.05$. A total of 218 oocytes were analyzed. Lack of vehicle-related effects was noticed (23/37, 62% vs 62/96, 65%, for oocytes cultured with or without the vehicle; $P> 0.05$). OTA caused a slight ($P>0.05$) reduction of the maturation rate (39/85, 46% vs 23/37, 62%, for exposed and controls, respectively). Instead, it affected oocyte bioenergetic status, as it reduced the rate of oocytes showing healthy perinuclear/pericortical mitochondrial distribution pattern (4/39, 10% vs 9/23, 39%, $P<0.05$). These data indicate that OTA, in the exposure conditions used in the present study,hinders nuclear and cytoplasmic maturation in prepubertal lamb oocytes.

[1] Malir et al. Ochratoxin A: 50 Years of Research, Journal of Toxins, 8:191, 2016. [2] Malir et al Ochratoxin A: Developmental and Reproductive Toxicity, Journal of Birth Defects Research (Part B), 98:493-502, 2013. [3] Martino et al. Supplementation with Nanomolar Concentrations of Verbascoside During in Vitro Maturation Improves Embryo Development by Protecting the Oocyte Against Oxidative Stress: A Large Animal Model Study,Journal of Reprod Toxicol, 65:204-211, 2016. [4] Huang FJ et al Effects of ochratoxin A on mouse oocyte maturation and fertilization, and apoptosis during fetal development, .Journal of Environ Toxicol, 31:724-735, 2016

This study was supported by Rep-Eat – H2020 MSCA-COFUND 2015.Grant Agreement No.713714