

ORAL COMMUNICATIONS

FROM GUT MICROBIOTA TO GONADS: BUTYRATE MITIGATES PFAS-PESTICIDE MIXTURE TOXICITY IN ZEBRAFISH SPERMATOGENESIS**M. Lombó, N. Baldoni, C. Giommi, O. Carnevali***Dept. of Life and Environmental Sciences, Polytechnic University of Marche, Ancona, Italy*

Endocrine-disrupting chemicals (EDCs) are ubiquitous contaminants that occur as environmental mixtures and reported to impair reproductive function. Glyphosate (GLY) and perfluorooctanoic acid (PFOA), commonly detected in water and food, contribute to chronic low-dose exposure in humans and wildlife. Conversely, postbiotics, especially short-chain fatty acids (SCFAs) generated by microbial fermentation of dietary fiber, are emerging as key regulators of reproductive physiology, with butyrate playing a promising role.

This study hypothesized that butyrate can mitigate the deleterious effects of a GLY+PFOA mixture on male reproduction using zebrafish as a model. Testes were cultured *ex vivo* in paired conditions: one testis per-animal served as control, while the contralateral testis was exposed to sodium butyrate (SB) (1 mM), GLY+PFOA (0.5 µg/L each; consistent with EU Drinking Water Directive limits for total pesticides and PFAS), or GLY+PFOA+SB at the previous concentrations. Spermatogenesis was assessed histologically, and histone acetylation (H3K9ac, H4K12ac) was evaluated in ha-

ploid and diploid germ cells through immunohistochemical analysis.

SB-treated testes closely resembled control ones. In contrast, GLY+PFOA exposure increased type B spermatogonia and reduced both the proportion and area of spermatozoa, indicating impaired germ cell differentiation. Notably, co-treatment with SB restored spermatozoa population to control values. At the epigenetic level, SB increased H3K9ac in testicular cells, whereas GLY+PFOA elevated H3K9ac selectively in diploid cells. Co-treatment with SB normalized H3K9ac levels to those of controls. H4K12ac was selectively increased in haploid cells by SB, while GLY+PFOA alone had no significant effect.

These findings evidenced the ability of GLY+PFOA mixture to alter spermatogenesis and indicated that butyrate can counteract the reprotoxic effects of EDC mixtures and suggest the necessity of further mechanistic and *in vivo* investigations.

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